PHYTOREMEDIATION OF SOILS CONTAMINATED BY OIL AND GAS DRILLING AND PRODUCTION OPERATIONS USING GRASS SPECIES

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Phytoremediation of Soils Contaminated By Oil and Gas Drilling and Production Operations Using Grass Species

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Soil contaminated by crude oil or drill cuttings poses a threat to ecosystem. The objective of this study was to test tolerance levels of grass species to drill cuttings and crude oil in seed germination and at the 5-leaf stage.

Sixty five grass species were screened for their tolerance to crude oil and drill cuttings at the germination stage. Two species were grouped as tolerant, 18 species as moderately tolerant, 27 species as moderately sensitive, and 18 species as sensitive to drill cuttings. In the test with crude oil, 28 species were classified as tolerant, 29 species as moderately tolerant, 6 species as moderately sensitive, and 2 species as sensitive.

Nine species were further tested at different contamination levels. Seed germination and seedling biomass of all species was reduced. Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.), showed the least reduction of germination and biomass when grown in contaminated soil. Thus, it is a potential species to be used in remediation of oil contaminated with hydrocarbons.

Seventy two grass species also were screened at 5-leaf stage for their tolerance to crude oil and drill cuttings. Thirteen species, among which seven are cereal crops, showed visual injury index less than 20 in a 0 to 100 scale, when grown in soil contaminated with drill cuttings. Of the grass species screened, grassy weeds ranked in the top one-third of biomass reduction with only yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult.) and foxtail barley (*Hordeum jubatum* L.) as exceptions.

Nine species were chosen to further test the growth and phytotoxicity at different levels of contamination. The responses of those species at mature stages were affected by growing conditions. Nevertheless, barley and yellow foxtail showed lower biomass reduction and phytotoxicity compared with the other species.

Using Fourier transform infrared spectroscopy to test the soil samples, it was found that concentrations of hydrocarbons in soil were reduced differently by different species. Annual ryegrass (*Lolium multiflorum* Lam.) and barley (*Hordeum vulgare* L.) showed the highest reduction of hydrocarbons from drill cuttings, while yellow foxtail and annual ryegrass showed the highest reduction of hydrocarbons from crude oil contamination.

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1. INTRODUCTION

The oil and gas industry is vital to the economy of North Dakota. More than 8,000 oil wells have been completed in western North Dakota's rugged prairie, which brought in $4 billion tax revenue since 2010 for the state (Kusnetz, 2014). In addition to the economic benefits provided to the state and its residents by a strong oil and gas exploration industry, environmental damages caused by accidental spilling of crude oil and fracking solution can cause long-term soil contamination.

1.1. Hydrocarbons

Hydrocarbons are organic compounds consisting entirely of hydrogen and carbon. There are two main categories, one with a benzene ring referred to as aromatic hydrocarbons, and the other is aliphatic hydrocarbons (Van Epps, 2006). Aliphatic hydrocarbons are further classified as saturated and unsaturated hydrocarbons. Saturated hydrocarbons also are called alkanes, and contain straight-chain alkanes (C_nH_{2n+2}) and cycloalkanes (C_nH_{2n}) with one or more carbon rings. Unsaturated hydrocarbons include alkenes (C_nH_{2n}) with a double bond between carbons, and alkynes (C_nH_{2n-2}) with one triple bond between carbons (Van Epps, 2006).

Hydrocarbons are of great importance to human beings as a major component of petroleum and chemical products. Petroleum is comprised of 84% hydrocarbons mostly alkanes, cycloalkanes and aromatic hydrocarbons (Van Epps, 2006). Saturated alkanes are refined into petrol containing 5- to 8-carbon molecules, and diesel fuel, kerosene and jet fuel containing 9- to 16-carbon molecules (Van Epps, 2006). Fuel oil and lubricating oil are refined from alkanes with more than 16 carbons. Paraffin wax is an alkane with approximately 25 carbons, and asphalt contains 35 or more (Van Epps, 2006). The remaining 16% of petroleum hydrocarbons are used as raw materials in the chemical industry, for instance, pharmaceuticals, solvents, fertilizers, pesticides, and plastics. There is almost an unlimited number of derivatives from hydrocarbons.

Many hydrocarbons are hazardous to the environment and can be toxic, mutagenic, and carcinogenic to humans. The U.S. EPA listed hydrocarbons as precursors of ground-level ozone. Hydrocarbons are emitted into the atmosphere primarily by incomplete fuel combustion, fuel evaporation, and other sources (Borden, 1994; Haritash and Kaushik, 2009). Accidental spills during oil production operations, refining, storage, and transportation are major sources of pollution to soil and water. Polycyclic aromatic hydrocarbons (PAHs) are among the most hazardous hydrocarbons that contribute to soil and marine hydrocarbon pollution. The most widely existing PAHs are naphthalene, phenanthrene, pyrene, fluoranthene, anthracene, and benzo (g,h,i) perylene. Some PAHs are also contained in creosote as wood preservatives, industrial wastes, and other petroleum products. Natural sources of hydrocarbons include *cyanobacteria* (Aislabie et al., 2004) and green algae (Matsumoto et al., 1996).

Hydrocarbons used in gasoline production have a lower boiling point (pentane, benzene), while those in creosote and coal tars have a higher boiling point (Borden, 1994). Most hydrocarbons are hydrophobic and capable of accumulating in high concentrations in soil and sediments. They are chemically and biologically stable (Campanella et al., 2002) that can withstand degradation in soil for up to 40 years. (Aislabie et al., 2004). As a contaminant, the fate of hydrocarbons in the environment is affected by physical dispersion, dilution, volatilization, chemical transformation, and biological degradation by soil microbes (Aislabie et al., 2004). The physical processes include partitioning, sorption/desorption which eventually reduce the mass of the contaminants (Aislabie et al., 2004; Ehlers and Loibner, 2006). Many common contaminants are liquids that, like oil, do not dissolve readily in water. Such liquids are known as non-aqueous phase liquids (NAPLs). Ultimately, they are transported either in bulk, as a non-aqueous liquid, in vapor, or in low concentrations in the aqueous phase (Fine et al., 1997).

Biodegradation of hydrocarbons by soil organisms depends on microbial, hydrocarbon, soil, and environmental factors (Borden, 1994). Microbial factors include the type, number, and metabolic capacity of the microorganisms. Hydrocarbon factors includes composition, amount, physico-chemical properties, and molecular structure of the hydrocarbons. Important soil properties influencing the fate of hydrocarbons are pH, water content, organic matter (OM) content, quantity and quality of nutrients, and electron acceptors. Finally, the environmental factors include temperature, precipitation, light etc. (Borden, 1994; Ehlers and Loibner, 2006).

1.2. Drill Cuttings

During an oil drilling process, drill cuttings are brought above ground and disposed after recycling the separated drill bit lubricating materials (drill mud). The composition of drill cuttings is complex and varies from site to site. The well size, drilling material, and muds used, environmental conditions, the mineralogy of the strata overlying the target reservoir, and the drilling techniques determine the composition of the drill cutting collectively (Al-Ansary and Al-Tabaa, 2007; Breuer et al., 2004). The drill cuttings from the Red Sea offshore oil production area were analyzed and 11% hydrocarbon and high concentrations of Cr, Zn, Ba, Pb, Cl were found (Al-Ansary and Al-Tabaa, 2007). The drill cuttings from the North Sea contained hydrocarbons as high as 22.4% and different levels of metals (Al-Ansary and Al-Tabaa, 2007). Drill cuttings usually are high in salts (KCl and NaCl). The pH of most drilling muds is maintained between 9.5 and 10.5 to suppress corrosion and control the solubility of calcium and magnesium components (Bourgoyne et al., 1986). Corrosion control additives typically include calcium carbonate ($CaCO_3$), caustic soda, white lime, and sodium acid pyrophosphate

($Na_2(HPO_4)_2$). The heavy metals found in drilling muds are known toxicants that persist in the environment and tend to accumulate in food chains. The four heavy metals, Cr, Ba, Pb, and Zn, occur in high concentrations in most drilling muds. Most drilling mud heavy metals are associated with barite and bentonite (Carls et al., 1995).

1.3. Hydrocarbon Contamination

In areas where oil and gas development is prevalent, air, water and soil can become contaminated with oil and gas waste and byproducts. Soil contamination at petroleum drilling and production sites is caused primarily by the intentional, accidental, and incidental discharge of drilling fluids, crude petroleum, and refined petroleum products (i.e. fuels and lubricants used in machinery and equipment). Hydraulic fracturing is a practice that may involve the injection of toxic chemicals into or close to ground water sources. Storm water runoff from drilling sites may also contribute to ground and surface water pollution. Soil contamination may occur from oil and gas industry wastes which contain petroleum hydrocarbons, metals, radioactive materials, salts and other toxic chemicals.

Despite the low probability, major spills may occur occasionally resulting in significant economic damage to the oil industry and environmental damage to the agricultural industry and nearby communities. The North Dakota Century Code 38-11.2-07 addresses the legal responsibilities of mineral developers in regards to water pollution. It states that the developer shall conduct or have conducted an inventory of water wells located within one-half mile of where subsurface mineral exploration activities are conducted if such exploration activities appear reasonably likely to encounter ground water, or within one mile of a subsurface mineral production site. The North Dakota Department of Agriculture's mediation service helps to assist surface owners and energy companies in resolving estate surface damages disputes. However, there are no remediation guidelines or programs established for contaminated water and lands. For example, the spill at Continental's well in Williams County was reported by the company on April 2, 2011, and cleanup work was not completed more than a month after the spill was reported.

1.4. Hydrocarbon Toxicity

Hydrocarbons are toxic to many living organisms. Hydrocarbon pollution causes inhibition of seed germination (Pena-Castro et al., 2006) and also plant growth (Aksmann et al., 2011; Freedman and Hutchinson, 1976).

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) from municipal waste incineration are extremely toxic (Tuppurainen et al., 1999). 2,3,7,8-tetrachlorodibenzo-p-dioxin is considered the most toxic congerner which causes human health problems, such as carcinogenesis, reproductive toxicity, immune dysfunction, hepatotoxicity, teratogenicity, and endocrine changes (Ishida et al., 2005).

1.5. Soil Contaminated by Hydrocarbons

Leakage and/or accidental spillage of hydrocarbons occurs all over the world wherever petroleum production, transportation and utilization occurs (Adebiyi and Afedia, 2011). Contaminated areas vary from tropical (Froehner, 2010), coastal marine (Liang, 2011), arid and semi-arid inlands (Zyakun, 2012), to Arctic and Antarctic regions (Aislabie et al., 2004). Water-soluble nutrients often become limited for plants in contaminated soil because of the hydrophobicity of hydrocarbons (Joner and Leyval, 2001; Kirk et al., 2005). Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}) were decreased by crude oil contamination, and total sulfur content was unaffected (Everett, 1978). The effect on phosphorous is not consistent. Everett (1978) reported an increase in available phosphorus in wet tundra soil contaminated by crude oil at 12 L m^{-2}, and

Aislabie et al., (2004) reported that total phosphorus was not affected by hydrocarbon contamination in Antarctic soil. As a result of the increase of total carbon content in hydrocarbon contaminated soil, a larger C/N ratio and reduction of bioavailability of nitrogen and phosphorus is usually observed. Nitrate depletion in hydrocarbon contaminated Antarctic soil was reported (Aislabie et al., 2004).

Soil organic matter (SOM) stimulates the fungi-bacteria-urease system at low total petroleum hydrocarbon (TPH) concentration and has a positive correlation with TPH (Guo et al., 2012). Zyakun et al. (2012) stated that consumption of SOM by microbial populations increased in the presence of hydrocarbons. A strong relationship was found between the presence of hydrocarbons and soil pH with the pH shifting to neutral after contamination (Everett, 1978). The effects of hydrocarbon contamination on soil moisture are still questionable (Aislabie et al., 2004). Water repellency and soil disaggregation have been developed from crude oil contamination (Roy and McGill, 1998). Hydrocarbon contaminated soil can be slightly hydrophobic, therefore, reducing its water retention (Everett, 1978). However, Balks et al. (2002) did not report any difference in moisture content between the contaminated and control soils. Reduction of soil surface albedo by hydrocarbon darkening caused an increasing of daily maximum surface temperature (Balks et al., 2002). The water infiltration rate (hydraulic conductivity) was reduced as a result of the formation of a hydrophobic film on soil particles, and its intensity was affected by texture, structure, and volume of oil spill (Everett, 1978).

Roy and McGill (1998) reported a reduction of microbes in crude oil-contaminated soil, which was at least two orders of magnitude lower than the earlier estimation in the same uncontaminated soil ten years before. Culturable yeasts were detected in hydrocarbon contaminated soil and a shift of predominant fungal species was observed, where *Phialophora* spp. were more abundant in hydrocarbon-contaminated soils, whereas *Geotrichum* and *Chrysosporium* dominated uncontaminated pristine soils (Aislabie et al., 2001). The number of hydrocarbon-degrading bacteria including *Rhodococcus* (for alkanes), *Acinetobacter*, *Pseudomonas* (aromatic substrates), and *Sphingomonas* (aromatic substrates) was elevated in hydrocarbon-contaminated soil (Aislabie et al., 1998).

1.6. Soil Contamination by Drill Cuttings

The common components in drill cuttings are hydrocarbons, salts, and sometimes, metals. Therefore, the most detrimental effect of drill cuttings in the soil is because of high electric conductivity (EC) and sodium adsorption ratio (SAR). The Colorado Oil and Gas Conservation Commission regulates the limits after drill cuttings application in soil, with EC less than 4.0 dS m^{-1}, SAR less than 12, and pH to 6 to 9 (Colorado Department of Public Health and the Environment, 1996).

1.7. Remediation of Hydrocarbon Contamination

Remediation of contaminated soils on or near the site of contamination is most economical and desirable. Engineering methods, such as thermal treatment (Campanella et al., 2003; Norris, et al., 1999), incineration (McKendrick and Mitchell, 1978), and mechanical cleanup, can cause more adverse environment impact, e.g. permafrost melting in Antarctic (Aislabie, 2004). Engineering methods also are expensive. Bioremediation is another option which involves the using of microbes that can degrade hydrocarbons. However, most of the microbes capable of degradation of hydrocarbons in soils are not tolerant to cold temperatures and require a long time to take effect (Eriksson et al., 2003).

1.8. Pytoremediation of Hydrocarbon Contamination

Phytoremedation is a process that uses plants tolerant to contaminant. This may be combined with different cultural practices, such as soil amendments (biochar, compost, liquid organic matter, and fertilizer) to remedy the contaminated sites. Rhizosphere is the soil area around roots with distinguishable properties from the surrounding soil. Rhizosphere is the primary focus of new technology that addresses phytoremediation (Joner and Leyval, 2003). Genetic engineering is also used for phytoremediation and biomonitoring. Recombinant P450 (cytochrome P450) genes, which can be induced by aryl hydrocarbon receptor (AhR), and recombinant AhR-mediated GUS reporter gene expression system have been transformed into plants for phytoremediation (Shimazu et al., 2011). In addition, the performance of vegetation and microbes can serve as indicators of the safety level of the land before it is used for food production and other purposes (Scelza et al., 2010).

The phytoremediation of petroleum hydrocarbons relies heavily on rhizodegradation, phytostabilization, and hydraulic control strategies. Some plants have shown great potential as remediation agents. Grass species have an extensive root system, which is the desired characteristic in rhizodegradation and phytostabilization. Alfalfa (*Medicago sativa* L.) and tall fescue *(Festuca arundinacea* Schreb.) roots have great affinity for naphthalene. Once established, those species may be able to absorb the contaminant, preventing it from leaching to subsurface water and causing further environmental contamination (Schwab et al., 1998).

Currently, there is a lack of information regarding the effects of soil functionality by hydrocarbons especially in cold regions like North Dakota. In the past, remediation efforts have relied heavily on engineering and stimulating soil microbial populations. The ability to use grass species to remediate contaminated sites would provide oil and gas exploration companies with a faster, safer and less expensive way to remove hydrocarbons from the soil.

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2. GERMINATION OF GRASS SPECIES AFFECTED BY DRILL CUTTINGS AND CRUDE OIL CONTAMINATION IN SOIL

2.1. Introduction

Phytoremediation is an effective way to remediate soil contamination due to petroleum-based operations, but it is site-specific. Such contamination includes crude oil hydrocarbons and drill cuttings. Hydrocarbons are organic compounds consisting entirely of hydrogen and carbon. Drill cuttings are broken bits of solid wastes removed from the borehole of oil or gas wells (Breuer et al., 2004) and the cuttings usually containing drill bit lubricating chemicals, significant amount of hydrocarbon, heavy metals, and water soluble salts (Al-Ansary and Al-Tabaa, 2007). The success of phytoremediation is affected by climate, temperature, precipitation, soil type, and plant species. Direct seeding is the most economical way to reclaim soils contaminated by oil drilling operations. One of the advantages of direct seeding is it can introduce vegetation quickly to contaminated soil and there is a need to have vegetation established in a short time window. Another advantage is that different species can be prescribed based on the list of species prior to the disturbance.

One of the major limitations in reclamation of soils contaminated by petroleum hydrocarbon or drill cuttings using plant species is seed germination failure (Vans Epps, 2006). The tolerance levels during plant seed germination are different among species and cultivars. Banks and Schultz (2005) reported that the germinations of lettuce (*Lactuca sativa* L.), millet (*Panicum miliaceum* L.), radish (*Raphanus sativus* L.), red clover (*Trifolium pratense* L.), and wheat (*Triticum aestivum* L.) decreased in soils contaminated by motor oil compared with uncontaminated soils. They also reported that lettuce was the most sensitive, while western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love), sunflower (*Heliantus annus* L.), safflower (*Carthamus tinctorius* L.), and white clover (*Trifolium repens* L.) were not significantly affected by crude oil contamination in soil. Besalatpour et al. (2008) reported that seed germination was reduced by 52 and 56% in tall fescue (*Festuca arundinacea* L.) and puccinellia (*Puccinellia distance* L.), respectively, by low levels of oil hydrocarbon, whereas canola (*Brassica napus* L.) seed germination was not affected. Seed germination was also delayed by petroleum hydrocarbon for tall fescue (Besalatpour et al., 2008), creeping bentgrass (*Agrostis stolonifera* L.), highland bentgrass (*Agrostis castellana* L.), sweet vernal grass (*Anthoxanthum odoratum* L.), black grass (*Alopecurus myosuroides* Huds.), rough bluegrass (*Poa trivialis* L.), fodder burnet (*Sanguisorba minor* ssp. *Muricata* L.), chewing's fescue (*Festuca rubra* ssp. *commutata* L.), and strong creeping red fescue (*Festuca rubra* ssp. *rubra* L.) (Adam and Duncan, 2002). Seed germination of lettuce, onion (*Allium cepa* L.) and tomato (*Lycopersicum esculen*tum L.) in fluoranthene contaminated soils was inhibited as the concentration of fluoranthene reached at 2, 5, 10 mg L^{-1} (Kummerova and Kmentova, 2004). Hong et al. (2009) tested the response of 55 South Korean wild plant species to polycyclic aromatic hydrocarbon (PAH) in soil at 0, 10, 30, 100, and 300 mg kg^{-1}. They grouped the plants into highly resistant, moderately resistant, moderate, moderately susceptible, susceptible, and highly susceptible based on their seed germination response. Plants in the *Caryophyllaceae* family were highly or moderately susceptible to PAH; plants in *Poaceae* family showed a wide spectrum in tolerance to PAH; plants in the *Fabaceae* family were moderately or highly resistant to PAH (Hong et al., 2009). Ertekin et al. (2011) tested five red clover cultivars and three white clovers with crude oil contamination at 1, 5, and 7% (V/V), and found that only one white clover cultivar germinated, whereas all of the red clover cultivars germinated (Ertekin et al., 2011).

During an oil drilling process, drill cuttings are brought above ground and disposed after recycling the separated drilling muds. The composition of drill cuttings is complex and varies from site to site. The well size, drilling material, mud used, environmental conditions, mineralogy of the strata overlying the target reservoir, and the drilling techniques determine the composition of the drill cutting collectively (Al-Ansary and Al-Tabaa, 2007; Breuer et al., 2004). The drill cuttings from the Red Sea offshore oil production area were analyzed, and 11% hydrocarbon levels and high concentrations of Cr, Zn, Ba, Pb, and Cl were found (Al-Ansary and Al-Tabaa, 2007). The drill cuttings from the North Sea contained hydrocarbons as high as 22.4% and different levels of metals (Al-Ansary and Al-Tabaa, 2007). There are only few studies on seed germination affected by drill cutting. Chaineau et al. (1996) reported the seed emergence of maize (*Zea mays* L.), wheat and pea (*Pisum sativum* L.) was not affected by the application of drill cuttings (pH 9.1 to 10.1, 10% fuel oil, and 11% Ca) at 15, 30, and 60 Mg ha^{-1} in the field. The germination of cowpea (*Vigna unguiculata* L.) and maize seeds were completely inhibited by the soil that was collected around a waste pit and might have been contaminated by drill cuttings from Kutchalli, Nigeria (Anoliefo et al., 2006). The drilling wastes from an active well site in Alberta, Canada, decreased the germination rate of alfalfa (*Medicago sativa* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), maize, and radish (Saint-Fort and Ashtani, 2014).

North Dakota has a long oil drilling history that dates back to the 1950s. Two major cycles of oil drilling have previously happened in North Dakota. One peaked in 1958 with 454 completions, and the other peaked in 1981 with 834 completions (Carlson, 1990). Another oil boom began about 2010. More than 8,000 wells have been completed in western North Dakota's rugged prairie, which brought in $4 billion tax revenue for the state since 2010 (Kusnetz, 2014).

In addition, other benefits include the employment opportunities and giving to charitable organization (Kusnetz, 2014). However, the impact of oil drilling on the environment is not negligible. Large amount of drilling waste is generated with the oil production. Drilling waste contains high concentrations of hydrocarbon, heavy metals, and salts. Sometimes, it has some radioactivity from the shale. Inappropriate disposal or treatment of the waste generated in oil production can cause water and soil contamination, as well as damage of vegetation and wildlife. The accidental spills of crude oil or petroleum can cause detrimental effects (Van Epps, 2006).

A total of 90 native and introduced grass species are commonly found in North Dakota. These species are used for field crops, forage crops, biofuel crops, conservation, and as natural habitat for wildlife, although some are considered as weeds. Some of the grass species have proved good species for reclaiming contaminated soil (Sedivec et al., 2011). Prairie grasses have great potential to be used in phytoremediation of hydrocarbon contaminated soil because they have a fibrous root system, which results in a large surface area for hydrocarbon-degrading microbes to colonize. Some of the root exudates also play an important role in oil hydrocarbon degradation. Aprill and Sims (1990) evaluated 80 prairie grasses for phytoremediation of PAH in soil and found the PAH disappearance from soil with vegetation was greater than unvegetated soil. Some of the grasses they included are found in North Dakota as well. Little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and switchgrass are native to North Dakota and used as forage. A seed mixture of western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), slender wheatgrass (*Agropyron trachycaulum* (Link) Malte.), tall wheatgrass (*Agropyron elongatum* (Host.) Beauv.), green needlegrass (*Nassella viridula* (Trin.) Barkworth *Stipa viridula* Trin.), sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.), and yellow sweetclover (*Melilotus officinalis* (L.) Lam.) was used to revegetate an abandoned coal strip mine in North Dakota, and slender wheatgrass was determined to be most suitable for the reclamation (Gardiner, 1993). Grass species tolerant to petroleum hydrocarbon and drill cuttings in North Dakota have not been reported. Currently, there are about 44 introduced grass species in North Dakota, such as Kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* Leyss.), and tall fescue (*Festuca arundinacea* Schreb.). They can establish quickly in native grassland and compete well with native species, and are considered as invasive species in North Dakota (Sedivec et al., 2011).

From the ecological point of view, all species are of great importance, and should be evaluated in the response to soil contamination by oil and gas drilling and production operation, as well as their potential use for phytoremediation. In this study, we focused on grass species because most of them have large fibrous root system ideal for hosting soil microbes which contribute to the most of actual hydrocarbons reduction. Another reason is that grass species are relatively easy to establish and maintain. Lastly, the majority of species used for soil reclamation in the oil and gas exploration areas in North Dakota are grasses (Rinella et al., 2012). Our research will add more information of grasses for use in remediation and reclamation to the previous work by Sedivec et al. (2011) which did not address soil contamination by drill cuttings and crude oil.

In addition to the importance of selecting tolerant species to use in soil reclamation, plant seed germination is also an important means of monitoring hydrocarbon contaminations and the existence of their derivatives in soil, such as phenanthrene (Scelza et al., 2010). Mechanisms of inhibition of seed germination by petroleum hydrocarbons include formation of an oil film around seeds as a physical barrier to both water and oxygen transfer (Adam and Duncan, 2002), phytotoxicity of water soluble molecules in petroleum (Henner et al., 1999), and blocking the mobilization of seed reserves as a result of inhibition of gibberellin activity (Kummerova and Kmentova, 2004). The mechanisms of the effects of drill cuttings on seed germination are not well understood. However, based on the complex composition of drill cutting, the mechanisms of inhibition of seed germination by petroleum hydrocarbons, heavy metals, and salinity may all apply.

The objective of this study was to evaluate seed germination of grass species affected by crude oil and drill cuttings. The inclusion of grass species was based on their importance to oil production areas of North Dakota, which are of different origins and usages, such as native vs. introduced, forage/crops vs. weeds, annual vs. perennial. A primary goal was to provide a list of grass species that are tolerant to those contaminants and can potentially be used to establish vegetation for phytoremediation and soil reclamation. The information may also be useful to understand potential impact of soil contamination by petroleum and drill cuttings on native habitat and seed banks.

2.2. Materials and Methods

2.2.1. Preliminary germination test

Sixty five grass species (including five cereal crops) that exist in the oil production areas of North Dakota, or are of great value in soil reclamation based on previous studies, were included for preliminary screening on seed germination affected by crude oil and drill cuttings. Seed sources are indicated in Table 2.1 and species described in (Table 2.2).

Table 2.1. Sources of seeds used in the preliminary screening for tolerance to drill cutting and crude oil contamination at the germination stage.

Company/Facility name	City	State	Abbreviations
Agassiz Seed & Supply	Fargo	ND	AGS
Aberdeen PMC†	Aberdeen	ID	ABD
Bismarck PMC	Bismarck	ND	BSM
Bridger PMC	Bridger	MT	BRD
Elstel Farm & Seed	Thomas	OK	EFS
Jacklin Seed Co.	Post Falls	ID	JKL
Knox city PMC	Knox City	TX	KXC
Los Lunas PMC	Los Lunas	NM	LLN
Millborn Seeds	Brookings	SD	MLB
North Dakota State University	Fargo	ND	NDSU
Prairie restoration Inc.	Princeton	MN	PRR
Pullman PMC	Pullman	WA	PLM
Rivard's TURF & FORAGE	Grand Forks	ND	RWD
SIMPLOT Jacklin seed division	Post Falls	ID	SPL
Tee-2-Green Corp.	Hubbard	OR	TTG
Twin City Seed Co.	Edina	MN	TCS
Upper Colorado Environmental Plant Center	Meeker	CO	UCEP

† Plant material center.

Table 2.2. Plant species used for the preliminary screening for germination tolerance to drill cuttings and crude oil.

Common name	Scientific name	Variety	Seed source[†]	Common name	Scientific name	Variety	Seed source
Kentucky bluegrass	*Poa pratensis* L.	Park	AGS	Western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Löve	Rodan	BSM
Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	Red River	EFS	Russian wildrye	*Elymus junceus* Fisch.	Mankota	BSM
Creeping bentgrass	*Agrostis stolonifera* L.	Penn 4	TTG	Sand dropseed	*Sporobolus cryptandrus* (Torr.) Gray	SD native	MLB
Colonial bentgrass	*Agrostis capillaris* L.	Alister	TTG	Desert wheatgrass	*Agropyron desertorum* (Fisch. ex Link) Schult.	Nordan	BSM
Strong creeping red fescue	*Festuca rubra* L. ssp. *rubra*	Navigator II	AGS	Siberian wheatgrass	*Agropyron fragile* (Roth) Candargy	Vavilov II	ABD
Buffalograss	*Buchloe dactyloides* (Nutt.) Engelm.	Bowie	RWD	Bluebunch wheatgrass	*Pseudoroegneria spicata* (Pursh) A. Löve	Anatone	ABD
Perennial ryegrass	*Lolium perenne* L.	Panther	RWD	Sand dropseed	*Sporobolus cryptandrus* (Torr.) Gray	Borden county	KXC
Kentucky bluegrass	*Poa pratensis* L.	Bewitched	TCS	Beardless wheatgrass	*Pseudoroegneria spicata* (Pursh) Love ssp. *inermis* (Scribn & Sm.) A. Löve	Whitmar	PLM
Hybrid crested wheatgrass	*Agropyron desertorum* (Fisch. ex Link) J.A. Schultes ×*Agropyron cristatum* (L.) Gaertn.	HyCrest	UCEP	Pubescent Intermediate wheatgrass	*Agropyron trichophorum*	Manska	MLB
Sheep fescue	*Festuca ovina* L.	Blue Ray	AGS	Sand bluestem	*Andropogon hallii* Hack.	Elida	LLS

(continues)

Table 2.2. Plant species used for the preliminary screening for germination tolerance to drill cuttings and crude oil. (continued)

Common name	Scientific name	Variety	Seed source[†]	Common name	Scientific name	Variety	Seed source
Annual ryegrass	*Lolium multiflorum* Lam.	VNS[‡]	AGS	Thickspike wheatgrass	*Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould	Sodar	MLB
Slender wheatgrass	*Elymus trachycaulus* (Link) Gould ex Shinners	Revenue	RWD	Canada bluegrass	*Poa compressa* L.	Cannon	MLB
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	Itasca	RWD	Maize	*Zea mays* L.	NDBS1011	NDSU
Weeping alkaligrass	*Puccinellia distans* (Jacq.) Parl.	Fults	RWD	Hard red spring wheat	*Triticum aestivum* L.	Glenn	NDSU
Timothy	*Phleum pratensis* L.	Climax	RWD	Hard red winter wheat	*Triticum aestivum* L.	Jerry	NDSU
Tall wheatgrass	*Agropyron elongatum* (Host.) Beauv.	Alkar	RWD	Oat	*Avena sativa* L.	Jury	NDSU
Orchardgrass	*Dactylis glomerata* L.	Potomac	RWD	Durum wheat	*Triticum durum* L.	Tioga	NDSU
Meadow brome	*Bromus biebersteinii* Roem.	Fleet	RWD	Barley	*Hordeum vulgare* L.	Pinnacle	NDSU
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	Pierre	RWD	Sweet corn	*Zea mays* L. var. *saccharata*	Synergy	NDSU
Canada wildrye	*Elymus Canadensis* L.	Mandan	RWD	Tall fescue	*Festuca arundinacea* Schreb.	Stonewall	JKL
Canada bluegrass	*Poa compressa* L.	Foothills	BRD	Idaho bentgrass	*Agrostis idahoensis* Nash	Golfstar	JKL
Creeping meadow foxtail	*Alopecurus arundinaceus* Poir.	Garrison	BRD	Foxtail barley	*Hordeum jubatum* L.	VNS	NDSU

(continues)

Table 2.2. Plant species used for the preliminary screening for germination tolerance to drill cuttings and crude oil. (continued)

Common name	Scientific name	Variety	Seed source†	Common name	Scientific name	Variety	Seed source
Basin wildrye	*Leymus cinereus* (Scribn. & Merr.) Löve	Trailhead	BRD	Witchgrass	*Panicum capillare* L.	VNS	NDSU
Prairie sandreed	*Calamovilfa longifolia* (Hook.) Scribn.	Goshen	BRD	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	VNS	NDSU
Thickspike wheatgrass	*Elymus lanceolatus* (Scribn. & J.G.Sm.) Gould	Critana	BRD	Johnsongrass	*Sorghum halepense* (L.) Pers.		NDSU
Sand bluestem	*Andropogon hallii* Hack.	Chet	MLB	Japanese brome	*Bromus japonicus* Thunb.	VNS	NDSU
Fairway crested wheatgrass	*Agropyron cristatum* (L.) Gaertn.	Douglas	ABD	Quackgrass	*Elymus repens* (L.) Gould	VNS	NDSU
Mammoth wildrye	*Leymus racemosus* (Lam.) Tzvelev	Volga	PLM	Downy brome	*Bromus tectorum* L.	VNS	NDSU
Switchgrass	*Panicum virgatum* L.	Forestburg	RWD	Barnyardgrass	*Echinochloa crus-galli* (L.) P. Beauv.	VNS	NDSU
Indiangrass	*Sorghastrum nutans* (L.) Nash	Tomahawk	RWD	Smooth crabgrass	*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.	VNS	NDSU
RS hybrid wheatgrass	*Elymus hoffmannii* Jensen & Asay	Saltlander	RWD	Proso millet	*Panicum miliaceum* L.	VNS	NDSU
Intermediate wheatgrass	*Agropyron intermedium* (Host.) Beauv.	Manifest	BSM	Fowl bluegrass	*Poa palustris* L.	VNS	PRR
Big bluestem	*Andropogon gerardii* Vitman	Bison	RWD				

† Seed source refers to Table 2.1.
‡Variety not stated (VNS).

2.2.1.1. Seed germination in soil containing drill cuttings

The soil used in this study was a sandy loam (Oye Hubert & Sons Construction, Fargo, ND) with pH of 6.79, electric conductivity (EC) of 0.235 dS m^{-1}, and bulk density of 1170 kg m^{-3}. The soil was air-dried and sieved to pass a 1-mm screen before use. Oil drill cuttings (Pioneer Energy Services Corp. San Antonio, TX) from Bakken oil fields in western North Dakota, had a sodium absorption ratio (SAR) of 47.7, EC of 5.0 dS m^{-1}, pH 9.8, and total petroleum hydrocarbon (TPH) 108100 mg kg^{-1}, and Ca, Mg, Mn, Na, Cl, and HCO_3^- were 502, 1150, 3.5, 8460, 6820, and 1810 mg kg^{-1}, respectively. The Fourier Transform Infrared (FTIR) spectrum of the drill cuttings is shown in Fig. 2.1.

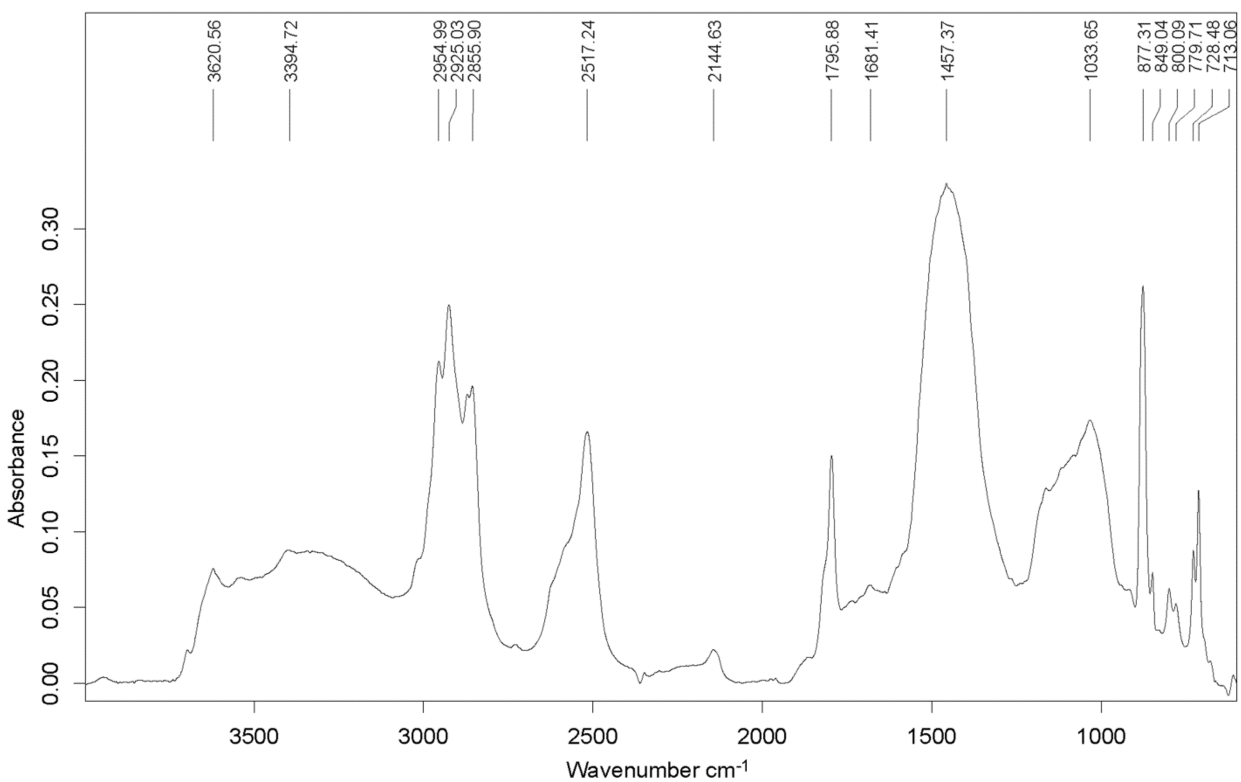

Figure 2.1. Absorbance spectrum of drill cuttings from Bakken oil fields in western North Dakota.

Soil contamination by drill cuttings was simulated by mixing 10 parts of uncontaminated soil with one part of drill cuttings on volumetric basis. Disposable sterile polystyrene Petri dishes measuring 100 mm ×15 mm were used for germination study. To each Petri dish, 30 cm^3 of uncontaminated or contaminated soil was added and pressed gently with a spatula. One hundred grass species seeds or fifty crop seeds (wheat, barley, maize, and sweet corn (*Zea mays* var. *saccharata*)) were placed in an individual Petri dish. The seeds were either covered or pressed to a depth equivalent to their seed size. The soil surface was gently pressed again to make good seed to soil contact prior to adding 13 mL distilled water, covered with lids, and sealed with parafilm.

Those species that require chilling treatment (ISTA, 1996) were seeded one week ahead of other species and kept in 4℃ for chilling treatment. After chilling treatment, all species were put in a growth room at temperature of 23℃ and 14-h photoperiod. The treatments were arranged in a randomized complete block design with three replicates. The blocks were arranged by potential temperature gradients from windows to the sidewalk.

2.2.1.2. Seed germination in soil containing crude oil

To simulate crude oil contamination, the uncontaminated soil was spiked with crude oil (Tesoro Refining & Marketing Co. San Antonio, TX) from Bakken oil fields in western North Dakota, which contains TPH of 99%, among which 1% N-hexane, 1.5% benzene, 0.1% naphthalene, and 0.1% xylene. The FTIR spectrum of the crude oil is shown in Fig. 2.2. One part of crude oil and 8.5 parts of soil was mixed at volumetric basis and was allowed to incubate for 1 week prior to use. The soil material was put into the above-described polystyrene Petri dishes using similar methods. The germination procedure was the same as in the drill cuttings study above.

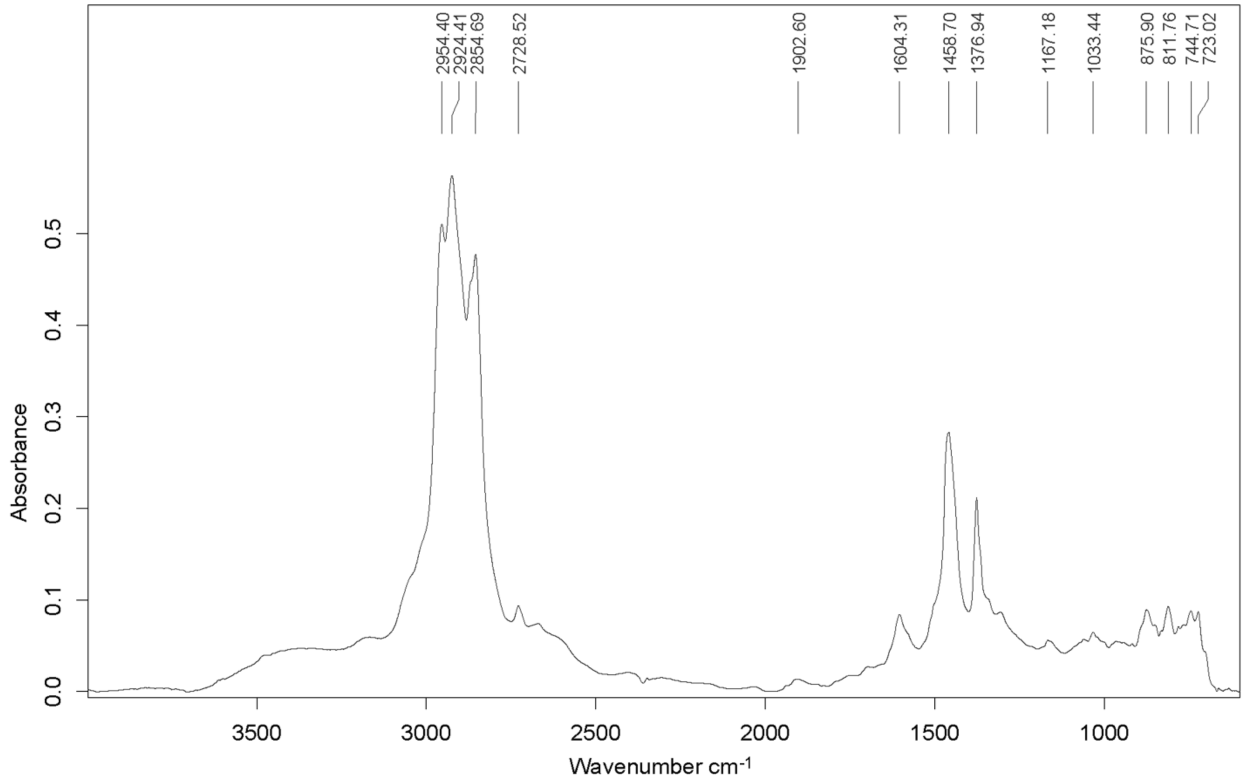

Figure 2.2. Absorbance spectrum of crude oil from Bakken oil fields in western North Dakota.

2.2.1.3. Determination of germination

Two weeks after all seeds were incubated in the growth room, germination was determined by counting the seedlings in each Petri dish. Only seedlings with essential structures (root system, shoot axis, and coleoptile) were counted. Both the normal seedlings (intact seedlings, seedlings with slight defects, and seedlings with secondary infection) and abnormal seedlings (damaged seedlings, deformed seedlings, and decayed seedlings) were included (ISTA, 1996).

The relative germination of each species was calculated using the germination in uncontaminated soil as 100%. Based on the germination reduction as compared with untreated control, the 65 species were grouped into sensitive (>75% reduction), moderately sensitive (50 to75% reduction), moderately tolerant (25 to 50% reduction), and tolerant (<25% reduction) to drill cuttings and crude oil, respectively. A similar grouping system was used by Hong et al. (2009). Plant germination and germination reduction data were tested with capability procedures in SAS for the normality of distribution Both the germination and relative germination data were analyzed with ANOVA using the general linear model in SAS (SAS Institute, 2013). Mean separation was done using F-protected Tukey test at 0.05 significance level. Replicates were considered random effects and treatments and species as fixed effects.

2.2.2. Seed germination affected by different concentration of drill cuttings and crude oil in soil

Nine species based on tolerance were selected for further evaluation on seed germination in response to different levels of drill cuttings and crude oil contamination in soil. Consideration was also given to different usages of grass species when including them for further evaluation such as forage, turfgrass, erosion control, and weeds. Efforts were also made to include some balance between introduced and native species. Finally, species that had germination less than 30% in the treated soil were not included.

2.2.2.1. Drill cuttings dose effect

The same top soil used in preliminary screening was used for this study. It was sieved to pass a 1-mm screen before use. To create different levels of contaminations, soil was mixed thoroughly with drill cuttings at concentration of 0, 0.05, 0.10, 0.15, and 0.20 $m^3\ m^{-3}$on volumetric bases. Nine plant species (strong creeping red fescue ‘Navigator II’, perennial ryegrass ‘Pather’, orchardgrass ‘Potomac’, buffalograss ‘Bowie’, little bluestem ‘Itasca’, witchgrass ‘variety not stated (VNS)’, sand dropseed ‘Borden county germplasm’, Johnsongrass ‘VNS’, and smooth crabgrass ‘VNS’) selected from the preliminary screening were used for the test. The germination test procedure was as used in the preliminary screening. The treatments were arranged as randomized complete block design with three replicates and repeated once.

2.2.2.2. Crude oil dose effect

The same top soil used above was used in this study. The soil was screened to pass 1-mm sieve prior to use. Different concentrations of crude oil at 0, 0.015, 0.030, 0.045, and 0.060 $m^3\ m^{-3}$ were created by mixing crude oil thoroughly with soil and incubated for 1 wk before use for germination test. The same nine species used for experiment 2.2.2.1 were used in this experiment. Germination test procedure was the same as explained in experiment 2.2.2.1. The treatments were arranged as randomized complete block design with three replicates and repeated once.

2.2.2.3. Data collection and analysis

Soil samples were taken the mixtures of different concentrations for the tests of pH and EC. The soil pH was tested in a 1:1 soil/ deionized water (V/V) suspension using a multi-parameter meter (HQ40d, Hach Company, Loveland, CO) and the EC was determined in a 1:5 soil/deionized water (V/V) extract using an EC meter (model 1054, VWR Scientific, Radnor, PA).

At the end of 2 wk germination period, seed germination percentages were determined and the biomass of seedlings from each Petri dish were determined after harvesting and oven-drying at 80°C for 24 h. The single plant biomass was calculated from the total biomass divided by the total number of seedlings in the Petri dish. Relative germination and biomass of each species were calculated using uncontaminated soil as 100%. Regression response of seed germination and biomass to different concentration of drill cuttings and crude oil was developed using regress procedures in SAS (SAS institute, 2013). The effective median concentration

(EC_{50}) is defined as the concentration at which 50% reduction occurs (Pena-Castro et al., 2006). The EC_{50} for drill cuttings and crude oil was calculated from the regression equation developed above. The data were subjected to ANOVA using general linear model in SAS with experiment and block as random variables, and species and concentrations of crude oil as fixed effects. Mean separation was done with F-protected Tukey test at 0.05 significance level.

2.3. Results and Discussion

2.3.1. Preliminary seed germination test

The data for germination were normally distributed. The species main effects in both drill cuttings and crude oil tests were significant (Table 2.3). Germination of all species was reduced by drill cuttings and crude oil. When expressed as percentage reduction of the germination in un-contaminated soil, drill cuttings and crude oil contamination caused significant germination reduction (Table 2.4). There was a significant interaction between the species and treatment, but this interaction is not discussed because the important parameter was percentage reduction. The significance in block effects indicated that the blocking was effective and variation within the block was reduced.

Table 2.3. ANOVA of seed germination as affected by drill cuttings and crude oil in the soil for the preliminary screening.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Block	2	0.067	25.2	<0.0001	0.126	31.9	<0.0001
Species (S)	64	0.127	48.1	<0.0001	0.186	47.4	<0.0001
Treatment (T)	1	7.898	2982.0	<0.0001	1.886	479.8	<0.0001
S × T	64	0.044	16.7	<0.0001	0.024	6.0	<0.0001
Error	258	0.003			0.003		
Total	389						

Table 2.4. ANOVA of seed germination reduction as affected by drill cuttings and crude oil in the soil for the preliminary screening.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Block	2	0.006	0.43	0.6516	0.017	1.51	0.2238
Species (S)	64	0.150	10.40	<0.0001	0.117	10.27	<0.0001
Error	128	0.014			0.011		
Total	194						

2.3.1.1. Seed germination in soil containing drill cuttings

The ranking of percentage reduction for each species as affected by drill cuttings is shown in Table 2.5. The reduction in germination ranged from 9.2% to 100%. Therefore, the concentration of drill cuttings used in the preliminary screening was able to separate different species. Two species were tolerant, 18 species were moderately tolerant, 27 species were moderately sensitive, and 18 species were sensitive.

Germination reduction of wheat and maize was lower than 37.5% and ranked lower than other grasses as moderate tolerant. This is in agreement with the report by Chaineau et al. (1996), in which corn and wheat germination was not affected by drill cuttings in soil at a similar TPH concentration used in this study. Seed size may be a factor since wheat and corn seeds are larger than most of other grass seeds. One of the mechanisms of inhibition of germination by petroleum hydrocarbon is coating of seeds with hydrophobic film and preventing water from entering (Adam and Duncan, 2002). Therefore, another reason for lower germination reduction in corn and wheat may be because the seeds are not covered with palea and lemma which makes water imbibition easier (Duclos et al., 2013; Maze et al., 1993).

Although the TPH content in the drill cuttings used in this study was comparable with many other reports ranging 4.2 to 22.4% (W/W) (Al-Ansary and Al-Tabaa, 2007; Breuer et al., 2004), direct comparison between the results and previous study is complicated because of the different hydrocarbon chemical components and salt content (Anoliefo et al., 2006). The drill cuttings used in this study had a high SAR and relative high EC. The effect of salinity on seed germination also needs to be considered when compared to previous studies that tested effect of drill cuttings on germination of grasses. For example, germination reduction of downy brome was 100%; this species is considered highly sensitive to salinity (Belnap et al., 2003). Similarly, slender wheatgrass and fairway crested wheatgrass showed 91 and 88.2% germination reduction in this study. These species are listed as salinity sensitive among 25 *Agropyron* species by Dewey (1960).

In contrast to *Poa* species which showed more than 80% reduction in germination, there is not a clear trend for the genus *Agropyron* and *Elymus*, which had multiple species in the preliminary screening and showed large variations within genus (Table 2.5). Since *Poa* species are generally more sensitive to salinity stress than many other cool-season turfgrasses (Dai et al., 2009) and *Agropyron* showed species differences within the genus (Dewey, 1960), additional testing with salinity and oil hydrocarbons to determine whether the reduction in germination in this study was attributed to salinity or to crude oil or both.

In addition to species, different genotypes within a species also showed different levels of tolerance to drill cuttings contamination. Little bluestem, sand dropseed, and Kentucky bluegrass are examples in this study (Table 2.5). Similar reports have been reported before on genotype differences to salinity tolerances (Qian et al., 2001; Horst and Taylor, 1983; Marcum, 2001; Robins et al., 2009), but no reports are available on drill cuttings effect.

To further detect the sensitivity of grass species to drill cuttings and crude oil, different levels of drill cuttings in soil need to be tested. From the preliminary results, nine species that

Table 2.5. Plant species germination and relative germination reduction (Red.) as affected by drill cuttings (DC).

Species	Control	DC	Red.	Species	Control	DC	Red.
		%				%	
Witchgrass	53.0	48.3	9.2	Switchgrass	24.3	9.3	61.4
Buffalograss	55.0	49.3	10.4	Barley	38.7	14.7	61.5
Big bluestem	46.7	33.7	25.7	RS hybrid wheatgrass	44.7	16.7	62.2
Hard red spring wheat	44.7	32.7	26.5	Indiangrass	55.3	20.7	63.4
Hard red winter wheat	41.7	28.0	33.1	Orchardgrass	68.0	24.0	64.4
Intermediate wheatgrass	76.7	50.3	33.9	Pubescent Intermediate wheatgrass	83.7	26.3	68.6
Durum wheat	33.0	21.0	35.6	Creeping meadow foxtail	51.7	15.7	69.3
Little bluestem†var.1	48.7	31.0	36.9	Bluebunch wheatgrass	11.7	3.3	69.9
Maize	52.0	32.3	37.5	Little bluestem†var.2	27.7	8.7	69.9
Thickspike wheatgrass	56.7	35.7	37.8	Tall wheatgrass	36.3	10.7	71.0
Sand bluestem	28.0	17.3	37.9	Siberian wheatgrass	68.0	19.0	71.6
Timothy	53.7	33.0	38.6	Tall fescue	45.7	12.3	73.1
Johnsongrass	35.7	21.7	39.0	Hybrid crested wheatgrass	50.0	13.0	74.0
Prairie sandreed	35.7	20.3	42.3	Idaho bentgrass	26.7	6.7	75.0
Meadow brome	87.3	46.3	46.6	Yellow foxtail	24.7	5.7	78.0
Barnyardgrass	47.7	24.7	46.6	Sweet corn	20.7	4.3	79.1
Basin wildrye	47.7	24.7	47.5	Desert wheatgrass	19.7	4.0	79.6
Large crabgrass	15.7	8.0	48.1	Creeping bentgrass	47.7	9.7	79.8
Strong creeping red fescue	85.7	43.3	49.6	Canada bluegrass	75.3	11.7	84.6
Sand dropseed‡var.1	57.7	28.3	50.0	Russian wildrye	25.0	3.7	86.4
Quackgrass	79.7	39.0	50.4	Mammoth wildrye	42.0	5.3	87.3
Perennial ryegrass	66.7	33.0	50.7	Fairway crested wheatgrass	10.0	1.3	88.2
Sideoats grama	28.7	13.7	51.4	Slender wheatgrass	88.0	8.0	91.0
Smooth crabgrass	35.7	17.0	52.0	Canada bluegrass	45.7	3.7	92.0
Colonial bentgrass	26.0	12.0	53.6	Sand dropseed ‡var.2	26.3	2.0	92.2
Oat	45.3	21.0	54.0	Kentucky bluegrass§var.1	64.3	5.0	92.3
Thickspike wheatgrass	67.7	31.0	54.5	Western wheatgrass	38.0	2.3	94.8
Annual ryegrass	42.7	19.7	55.6	Weeping alkaligrass	70.0	3.7	95.0
Sheep fescue	53.0	22.7	57.5	Kentucky bluegrass§var.2	42.7	0.3	99.3

(continues)

Table 2.5. Plant species germination and relative germination reduction (Red.) as affected by drill cuttings (DC). (continued)

Species	Control	DC	Red.	Species	Control	DC	Red.
	%				%		
Beardless wheatgrass	27.3	11.3	58.6	Canada wildrye	22.3	0.0	100.0
Fowl bluegrass	33.7	12.7	60.2	Foxtail barley	79.7	0.0	100.0
Japanese brome	50.0	18.7	60.9	Downy brome	57.7	0.0	100.0
Proso millet	20.0	7.7	60.9				
$HSD_{0.05}$¶	17.5	17.5	41.5	$HSD_{0.05}$¶	17.5	17.5	41.5

† Little bluestem var.1 is‘Itasca’; Little bluestem var.2 is ‘Bad land ecotype’.

‡ Sand dropseed var.1 is‘Borden county germplasm’; Sand dropseed var.2 is‘SD native’.

§ Kentucky bluegrass var.1 is ‘Park’; Kentucky bluegrass var.2 is‘Bewitched’.

¶ Tukey’s Studentized Range (HSD) at the 0.05 probability level.

had less than 65% germination reduction and representing different levels of tolerance to drill cuttings were selected.

2.3.1.2. Seed germination in soil containing crude oil

Preliminary screening of grass germination affected by crude oil contamination is shown in Table 2.6. The reduction in germination ranged from 4.3 to 100%. Using the same scale as in drill cuttings treatment, 28 species were tolerant, 29 species moderate-tolerant, 6 species moderate-sensitive, and 2 species sensitive. Like the responses to drill cuttings, cereal crops showed relative low germination reduction (<20%). Unlike in the drill cuttings, ‘Park’ and ‘Bewitched’ Kentucky bluegrass showed less germination reduction (17.6 and 40.3%) with the crude oil treatment and were tolerant and moderately tolerant instead of sensitive, suggesting that lack of salinity tolerance of this species played an important role in the lower tolerance to drill cuttings. Therefore, there was a better separation of Kentucky bluegrass varieties in response to crude oil treatment. Weeping alkaligrass had 64.7% germination reduction (Table 2.6) with the crude oil treatment and had 95% germination reduction (Table 2.5) with the drill cuttings treatment, indicating salt and other factors added to the inhibition due to hydrocarbon. Downy brome and foxtail barley had more than 95% germination reduction in both drill cuttings and crude oil, indicating hydrocarbons were primarily responsible for the reduction. Slender wheatgrass and hybrid crested wheatgrass showed more than 70% germination reduction in drill cuttings treatment (Table 2.5) but less than 8% germination reduction in crude oil treatment (Table 2.6), indicating that these two species are tolerant to petroleum hydrocarbon but not to the added salinity that drill cuttings have.

Table 2.6. Plant species germination and relative germination reduction (Red.) as affected by crude oil (Oil).

Species	Control	Oil	Red.	Species	Control	Oil	Red.
	———	% ———			———	% ———	
Sand dropseed†var.1	57.7	55.0	4.3	Buffalograss	56.7	38.7	31.9
Basin wildrye	49.3	46.7	5.4	RS hybrid wheatgrass	44.7	30.3	32.5
Slender wheatgrass	74.7	70.3	6.2	Sweet corn	20.7	14.0	32.6
Pubescent Intermediate wheatgrass	80.3	75.0	6.7	Smooth crabgrass	36.0	24.0	33.5
Hybrid crested wheatgrass	60.0	55.3	7.7	Proso millet	20.0	12.7	33.6
Oat	42.0	38.3	9.3	Colonial bentgrass	29.7	19.7	33.7
Hard red spring wheat	44.7	40.3	9.6	Large crabgrass	15.7	10.3	34.0
Japanese brome	50.0	45.0	9.9	Switchgrass	22.7	15.0	34.6
Sheep fescue	53.0	47.7	10.4	Sand dropseed†var.2	26.3	17.0	35.7
Witchgrass	53.0	47.3	10.4	Strong creeping red fescue	85.7	55.0	35.8
Maize	52.0	44.7	14.0	Orchardgrass	64.7	40.7	37.1
Creeping bentgrass	47.7	40.7	14.8	Intermediate wheatgrass	76.7	48.0	37.2
Thickspike wheatgrass	67.7	57.7	14.9	Perennial ryegrass	66.7	41.7	37.7
Hard red winter wheat	41.7	35.3	15.2	Siberian wheatgrass	71.3	43.7	38.5
Durum wheat	33.0	27.3	17.4	Bluebunch wheatgrass	11.7	7.0	39.1
Kentucky bluegrass‡var.1	64.3	53.0	17.6	Timothy	53.7	32.7	39.8
Barnyardgrass	47.7	38.7	17.6	Kentucky bluegrass‡var.2	38.7	23.0	40.3
Little bluestem††var.1	48.7	39.7	18.9	Prairie sandreed	35.7	21.7	41.1
Barley	38.7	31.3	18.9	Little bluestem††var.2	24.3	14.0	42.2
Johnsongrass	36.3	29.7	19.4	Thickspike wheatgrass	76.0	43.3	42.9
Sand bluestem	35.3	28.0	20.7	Tall fescue	45.7	26.7	43.0
Creeping meadow foxtail	48.7	39.0	21.1	Tall wheatgrass	30.7	17.0	43.8
Yellow foxtail	24.7	19.3	21.5	Canada bluegrass	39.0	22.0	44.0
Quackgrass	79.7	63.0	22.0	Idaho bentgrass	26.7	14.7	45.0
Indiangrass	55.3	43.0	22.1	Russian wildrye	25.0	12.3	51.5
Sideoats grama	22.0	17.0	22.4	Beardless wheatgrass	27.3	13.0	52.1
Annual ryegrass	42.0	32.7	22.7	Weeping alkaligrass	70.0	24.0	64.7
Meadow brome	87.3	67.3	23.1	Fairway crested wheatgrass	12.3	4.3	66.0

(continues)

Table 2.6. Plant species germination and relative germination reduction (Red.) as affected by crude oil (Oil). (continued)

Species	Control	Oil	Red.	Species	Control	Oil	Red.
	———	%	———		———	%	———
Mammoth wildrye	42.0	31.0	25.7	Canada wildrye	22.3	7.0	68.3
Western wheatgrass	33.3	24.7	27.3	Desert wheatgrass	19.7	5.0	74.9
Big bluestem	51.0	36.3	29.0	Foxtail barley	79.7	3.7	95.5
Fowl bluegrass VNS	33.0	24.3	29.2	Downy brome	54.7	0.0	100.0
Canada bluegrass	75.3	51.7	31.0				
$HSD_{0.05}$ ¶	21.3	21.3	36.9	$HSD_{0.05}$¶	21.3	21.3	36.9

† Sand dropseed var.1 is ‘SD native’; Sand dropseed var.2 is‘Borden county germplasm’.
‡ Kentucky bluegrass var.1 is ‘Park’; Kentucky bluegrass var.2 is ‘Bewitched’.
§ Little bluestem var.1 is ‘Itasca’; Little bluestem var.2 is ‘Bad land ecotype’.
¶ Tukey’s Studentized Range (HSD) at the 0.05 probability level.

Crude oil components were not the same as drill cuttings' components, and different hydrocarbons have different volatile properties and may contribute differently to the germination effect. Using diesel as hydrocarbon treatment, Adam and Duncan (2002) found that annual ryegrass and sheep fescue were more tolerant than orchardgrass at 50 g kg^{-1}, which is in agreement with the findings from this study. However, creeping bentgrass and quackgrass were ranked more sensitive than strong creeping red fescue by Adam and Duncan (2002), while in this study, they were more tolerant than strong creeping red fescue (Table 2.6). Using only PAH treatments in soil, Hong et al. (2009) ranked downy brome as highly susceptible and yellow foxtail as moderately susceptible. Similar results were observed in this study. However, Japanese brome was ranked as highly susceptible to PAH by Hong et al. (2009), while it was ranked tolerant to crude oil in this study. Tall fescue was ranked highly tolerant to PAH by Hong et al. (2009), but moderately sensitive to crude oil in this study. In addition to variety differences, different hydrocarbon effects will need to be evaluated.

Based on responses to drill cuttings and crude oil at the preliminary screening concentration, nine species were selected for further testing different concentrations of both drill cuttings and crude oil (Table 2.7).

2.3.2. Seed germination affected by different concentration of drill cuttings and crude oil in the soil

2.3.2.1. Drill cuttings dose effect

Both species and drill cuttings concentration had significant effect on seed germination (Table 2.8). The effects also depended on the species as significant interactions between species and drill cuttings concentrations were detected. Similar results were found for seedling biomass (Table 2.8).

Table 2.7. Germination and germination reduction (Red.) of selected plants species from the screening study used for the dose effect of both drill cuttings (DC) and crude oil (Oil) on seed germination.

Species	Control	DC	Red.	Control	Oil	Red.
	%			%		
Witchgrass	53.0	48.3	8.9	53.0	47.3	10.8
Buffalograss	55.0	49.3	10.4	56.7	38.7	31.7
Little bluestem†	48.7	31.0	36.3	48.7	39.7	18.5
Johnsongrass	35.7	21.7	39.2	36.3	29.7	18.2
Strong creeping red fescue	85.7	43.3	49.5	85.7	55.0	35.8
Sand dropseed‡	57.7	28.3	51.0	26.3	17.0	35.4
Perennial ryegrass	66.7	33.0	50.5	66.7	41.7	37.5
Smooth crabgrass	35.7	17.0	52.4	36.0	24.0	33.3
Orchardgrass	68.0	24.0	64.7	64.7	40.7	37.1

† Little bluestem 'Itasca'.

‡ Sand dropseed 'Borden county germplasm'.

Table 2.8. ANOVA of the reduction of seed germination and seedling biomass of nine grass species as affected by drill cuttings in the soil.

		Germination			Biomass		
Source of variation	df	MS	F	Pr>F	MS	F	Pr>F
Exp	1	0.386	9.5	0.0186	0.652	4.9	0.0500
Block within Exp	4	0.019	3.0	0.0213	0.057	6.9	<0.0001
Species (S)	8	0.391	12.1	0.0010	0.379	10.5	0.0016
Concentration (C)	4	3.368	421.5	<0.0001	4.696	87.3	0.0004
S × C	32	0.117	9.2	<0.0001	0.046	6.5	<0.0001
Exp × S	8	0.032	2.6	0.0278	0.036	5.1	0.0004
Exp× C	4	0.008	0.6	0.6428	0.054	7.7	0.0002
Exp × S × C	32	0.013	1.9	0.0038	0.007	0.9	0.7034
Error	176	0.007			0.008		

EC_{50} was defined as the effective concentration of drill cuttings or crude oil that caused 50% reduction in germination or biomass production in this study. The EC_{50} of nine grass species is shown in Table 2.9. Reduction in germination was also accompanied by reduction in biomass per seedling, and the EC_{50} for biomass is shown in Table 2.10. Witchgrass and buffalograss ranked on the top of nine species based on EC_{50} in agreement with the results from the preliminary study. However, not all species ranked the same as in the preliminary germination test indicating other parameters may also be needed when comparing the response of different species.

The slope of simple linear regression equation provided additional information to compare the response among species (Tables 2.11 and 2.12). The negative sign of slope indicated reduction of germination and biomass as the concentration increased. The absolute value of the slope indicated sensibility. However, the ranking was masked by the salinity effect (Fig. 2.3) since the effects of hydrocarbon and salinity are confounded. Both salinity and hydrocarbons increased as the concentration of drill cuttings increased.

Table 2.9. Germination of nine grass species affected by different concentrations of drill cuttings in the soil.

Species	EC_{50}†
	——$m^3\ m^{-3}$——
Buffalograss	N/A‡
Witchgrass	0.14
Smooth crabgrass	0.14
Perennial ryegrass	0.12
Johnsongrass	0.12
Sand dropseed	0.10
Little bluestem	0.10
Orchardgrass	0.10
Strong creeping red fescue	0.09

†EC_{50} is the effective concentration at which 50% of reduction in germination occurred.
‡N/A, less than 50% reduction at the highest concentration in this study was observed.

Table 2.10. Biomass of nine grass species affected by different concentrations of drill cuttings in the soil.

Species	EC_{50}†
	——$m^3\ m^{-3}$——
Buffalograss	N/A‡
Orchardgrass	0.13
Sand dropseed	0.11
Johnsongrass	0.10
Strong creeping red fescue	0.09
Perennial ryegrass	0.08
Witchgrass	0.07
Little bluestem	0.06
Smooth crabgrass	0.04

† EC_{50} is the effective concentration at which 50% of reduction in biomass occurred.
‡ N/A, less than 50% reduction at the highest concentration in this study was observed.

Table 2.11. Germination (y) (%) of nine grass species in response to drill cuttings concentration (x) ($m^3\ m^{-3}$) in the soil.

Species	Equation	r^2†
Buffalograss	y = 50.8 - 69.3x	0.42
Smooth crabgrass	y = 30.2 - 103x	0.66
Johnsongrass	y = 43.1 - 155x	0.67
Witchgrass	y = 58.2 - 202.7x	0.82
Little bluestem	y = 45.9 - 204.7x	0.78
Perennial ryegrass	y = 73.4 - 322x	0.82
Strong creeping red fescue	y = 72.7 - 383.3x	0.84
Sand dropseed	y = 90.4 - 435.3x	0.91
Orchardgrass	y = 92.0 - 457x	0.90

† Coefficient of determination (r^2) for germination as affected by the drill cuttings concentration in the soil.

Table 2.12. Biomass (y) (mg plant^{-1}) of seedling of plant species responses to drill cuttings concentration (x) (m^3 m^{-3}) in the soil.

Species	Equation	r^2†
Sand dropseed	y = 0.22 - 0.81x	0.61
Witchgrass	y = 0.28 - 1.12x	0.52
Buffalograss	y = 0.98 - 2.30x	0.77
Strong creeping red fescue	y = 0.47 - 2.34x	0.89
Orchardgrass	y = 0.62 - 2.53x	0.89
Smooth crabgrass	y = 0.58 - 2.89x	0.59
Perennial ryegrass	y = 0.67 - 2.92x	0.83
Johnsongrass	y = 1.58 - 5.96x	0.78
Little bluestem	y = 1.32 - 6.23x	0.75

† Coefficient of determination (r^2) of the biomass as affected by the drill cuttings concentration in the soil.

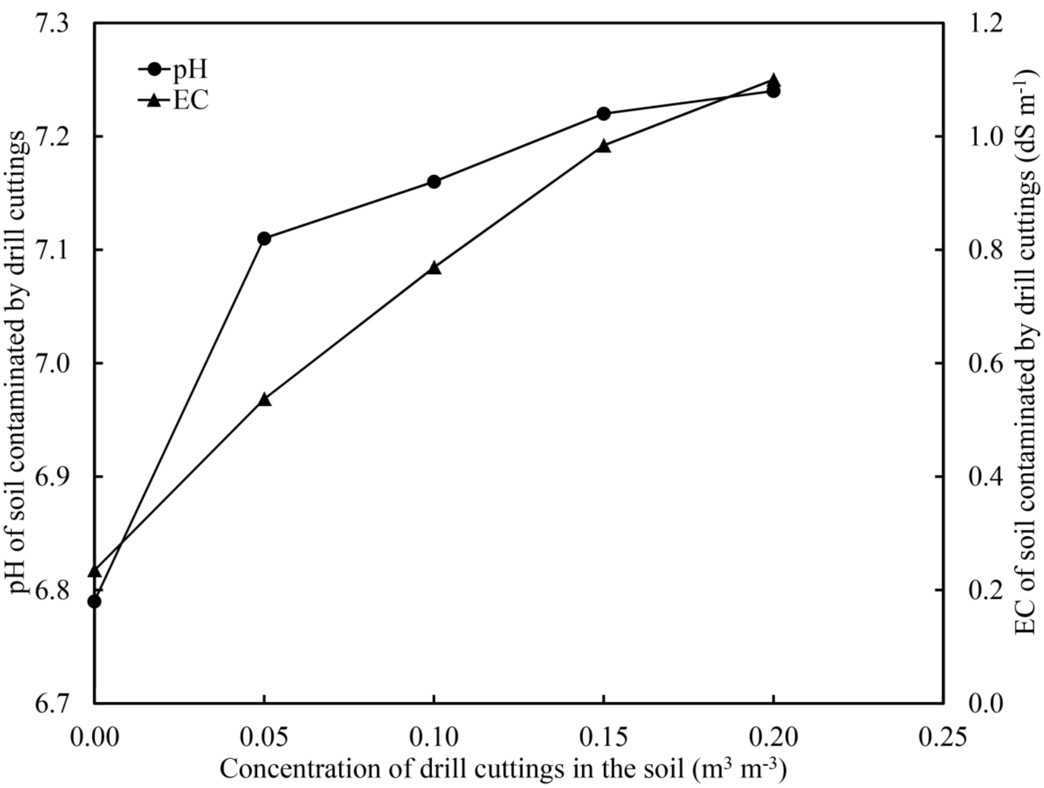

Figure 2.3. pH and electric conductivity (EC) of the soil affected by drill cuttings.

As the most drill cutting tolerant species, buffalograss had both the lowest germination reduction and highest absolute values at the highest contamination level in both germination and biomass (Fig. 2.4 and 2.5). Therefore, buffalograss has potential to be grown in drill cuttings contaminated soils for remediation and reclamation purposes, especially for it is value as a native species across the Great Plains. On the other hand, creeping red fescue showed high reduction and the lowest absolute value in germination and biomass, making it less desirable for phytoremediation of drill cuttings contaminated soils, despite the fact that it is recommended as a very good low maintenance turfgrass in the Midwest (Watkins et al., 2011).

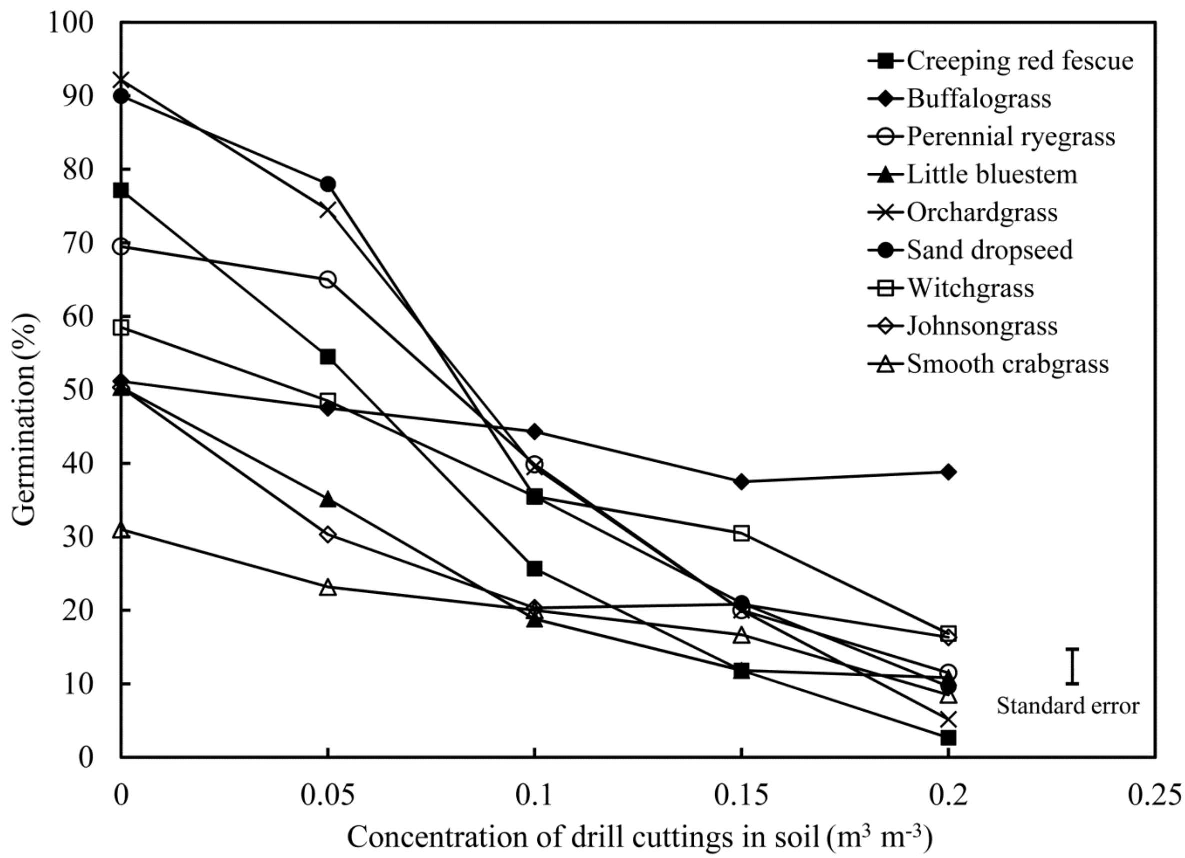

Figure 2.4. Seed germination of nine grass species responses to drill cuttings in the soil.

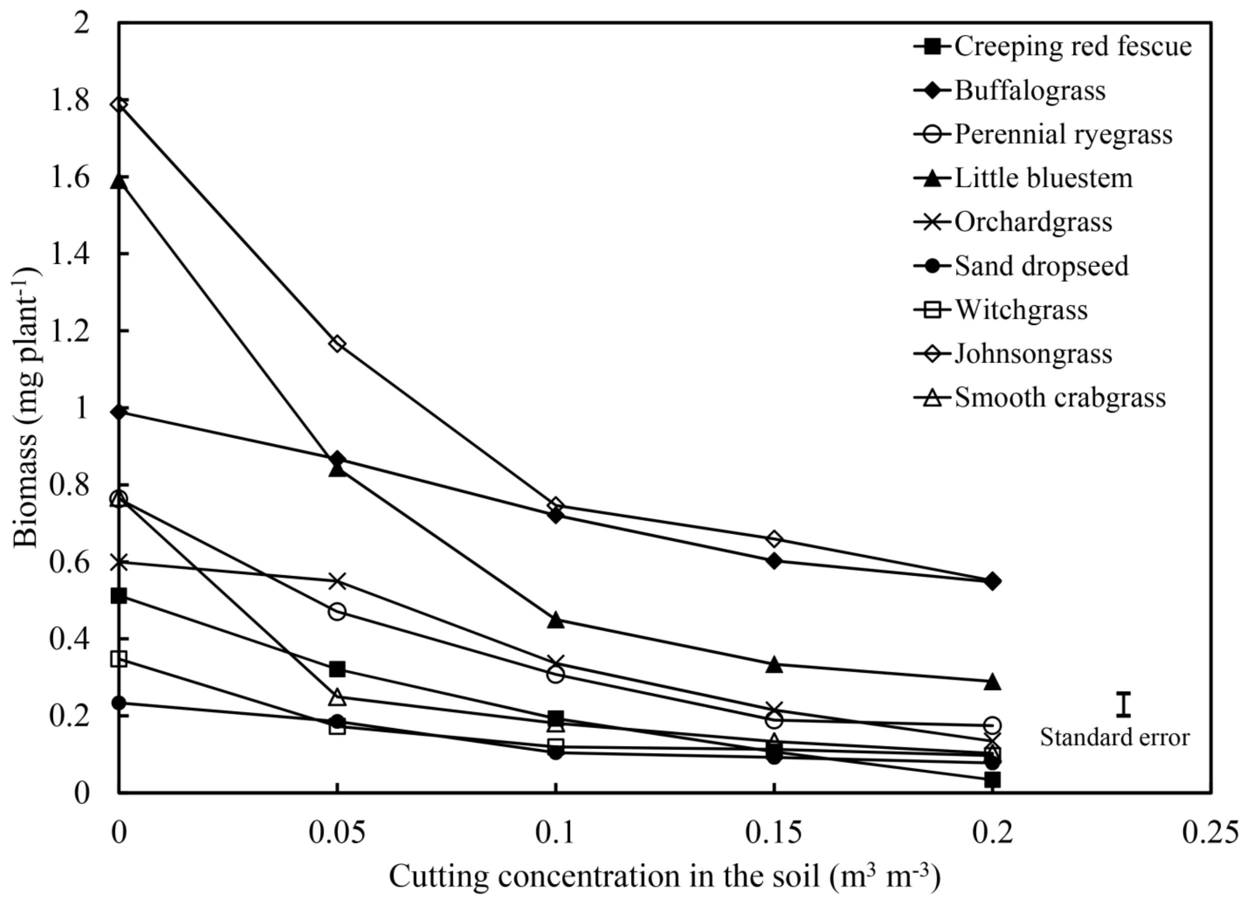

Figure 2.5. Biomass of nine grass species responses to drill cuttings in the soil.

<u>2.3.2.2. Crude oil dose effect</u>

Seed germination was significantly affected by species and crude oil concentrations in soils (Table 2.13). The interaction between species and crude oil concentration indicated the sensitivity those species may be different at different concentrations of crude oil levels. Similar results were found for the seedling biomass at the end of germination test (Table 2.13).

The EC_{50} of germination and biomass ranked nine species in a similar order (Tables 2.14 and 2.15). Buffalograss, sand dropseed, and orchardgrass ranked on the top as more tolerant species, whereas smooth crabgrass and little bluestem were ranked as more sensitive. The slightly different ranking based on germination and biomass may be caused by different sensitivity of physiological process during seed germination and seedling development to the components in the oil. Those include different volatility of different components and conversion of hydrocarbon into different chemicals in the soil (Chaineau et al., 1996). Similar phenomena was reported by Hong et al. (2009) in study of germination and growth responses of different

Table 2.13. ANOVA of the reduction of seed germination and seedling biomass of nine grass species affected by crude oil concentrations in the soil.

		Germination			Biomass		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Exp	1	0.203	3.4	0.1069	0.362	1.4	0.2735
Block within Exp	4	0.038	4.7	0.0013	0.179	14.6	<0.0001
Species (S)	8	2.086	69.9	<0.0001	0.575	8.8	0.0030
Concentration (C)	4	2.549	317.6	<0.0001	4.015	101.5	0.0030
S × C	32	0.119	15.6	<0.0001	0.073	6.3	<0.0001
Exp × S	8	0.030	3.9	0.0025	0.066	5.7	0.0002
Exp× C	4	0.008	1.1	0.3957	0.040	3.4	0.0192
Exp × S × C	32	0.008	0.9	0.5722	0.012	0.9	0.5592
Error	176	0.008			0.012		

Table 2.14. Germination of nine grass species affected by different concentrations of crude oil in the soil.

Species	EC_{50}†
	$m^3\ m^{-3}$
Sand dropseed	N/A‡
Buffalograss	0.10
Orchardgrass	0.05
Johnsongrass	0.05
Strong creeping red fescue	0.04
Perennial ryegrass	0.04
Little bluestem	0.04
Witchgrass	0.03
Smooth crabgrass	0.03

† EC_{50} is the effective concentration at which 50% of reduction in germination occurred.
‡ N/A, less than 50% reduction at the highest concentration in this study was observed.

Table 2.15. Biomass of nine grass species affected by different concentrations of crude oil in the soil.

Species	EC_{50}†
	——$m^3\ m^{-3}$——
Buffalograss	0.08
Orchardgrass	0.08
Sand dropseed	0.07
Strong creeping red fescue	0.04
Perennial ryegrass	0.04
Witchgrass	0.04
Johnsongrass	0.03
Little bluestem	0.03
Smooth crabgrass	0.03

† EC_{50} is the effective concentration at which 50% of reduction in biomass occurred.

Ranking of species using EC_{50} of crude oil concentrations was different from that found in the preliminary germination test indicating other parameters also will be needed in comparing the response of different species. The slope of simple linear regression equations provided useful information (Tables 2.16 and 2.17).

Table 2.16. Germination (y) (%) of nine grass species responses to crude oil concentration (x) ($m^3\ m^{-3}$) in the soil.

Species	Equation	r^2†
Sand dropseed	$y = 85.5 - 66.7x$	0.03
Buffalograss	$y = 50.6 - 243.3x$	0.34
Johnsongrass	$y = 46.0 - 443.3x$	0.71
Smooth crabgrass	$y = 31.5 - 587.8x$	0.83
Little bluestem	$y = 50.5 - 662.2x$	0.86
Witchgrass	$y = 61.4 - 1042.2x$	0.94
Orchardgrass	$y = 98.5 - 1095.6x$	0.75
Strong creeping red fescue	$y = 84.6 - 1160x$	0.80
Perennial ryegrass	$y = 77.2 - 1171.1x$	0.87

† Coefficient of determination (r^2) of the germination affected by the crude oil concentration in the soil.

Table 2.17. Biomass (y) (mg plant^{-1}) of nine grass species responses to crude oil concentration (x) (m^3 m^{-3}) in the soil.

Species	Equation	r^2†
Sand dropseed	y = 0.27 - 2.4x	0.49
Witchgrass	y = 0.33 - 3.3x	0.42
Orchardgrass	y = 0.67 - 4.7x	0.50
Strong creeping red fescue	y = 0.55 - 7.3x	0.82
Buffalograss	y = 1.05 - 7.6x	0.65
Perennial ryegrass	y = 0.79 - 10.2x	0.82
Smooth crabgrass	y = 0.75 - 14.0x	0.82
Johnsongrass	y = 1.60 - 21.2x	0.83
Little bluestem	y = 1.51 - 21.3x	0.79

† Coefficient of determination (r^2) of the biomass affected by the crude oil concentration in the soil.

The negative sign of the slope indicated reduction of germination and biomass as the crude oil concentration increased. The absolute value of the slope indicated sensibility. The ranking of species based on the slopes of simple linear regression equations (Table 2.16) was similar to the ranking in the preliminary experiment with crude oil. Unlike the drill cuttings experiment, soil pH and EC were not significantly changed as crude oil concentration increased (Fig. 2.6). Therefore, we concluded that the crude oil was mainly responsible for the effects. Buffalograss and johnsongrass showed lower germination and biomass reduction compared with other species at the highest contamination level (Figs 2.7 and 2.8).

Therefore these two species are potentially useful for phytoremediation and reclamation of crude oil contaminated soil. However, johnsongrass is listed as noxious invasion weed in many states (Gordon et al., 2011) and it does not grow in North Dakota because it is not winter hardy. Sand dropseed showed the lowest reduction in germination as affected by crude oil but the biomass at the end of germination was much lower than buffalograss and johnsongrass. Further evaluation of the growth of sand dropseed in crude oil contaminated soils is necessary.

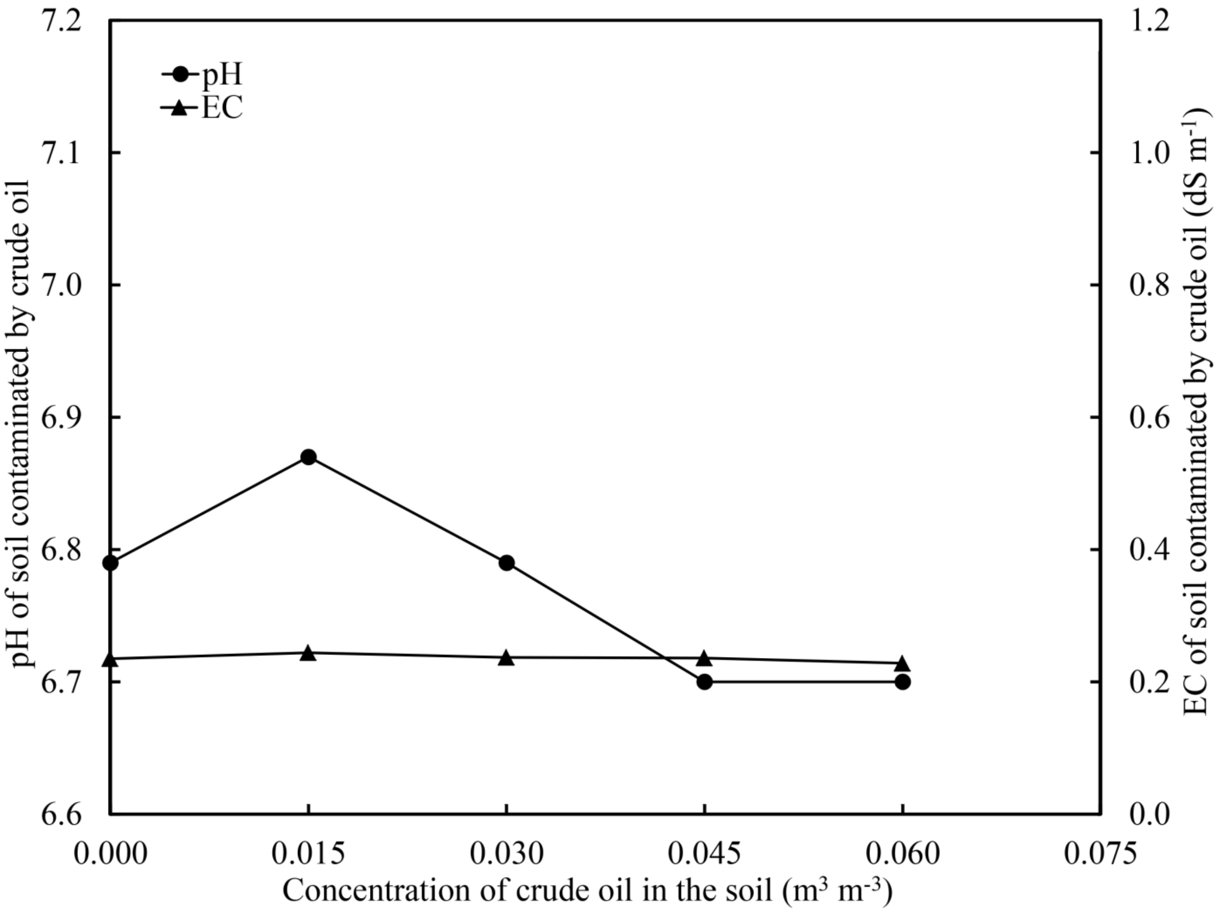

Figure 2.6. pH and electric conductivity (EC) of the soil contaminated by crude oil.

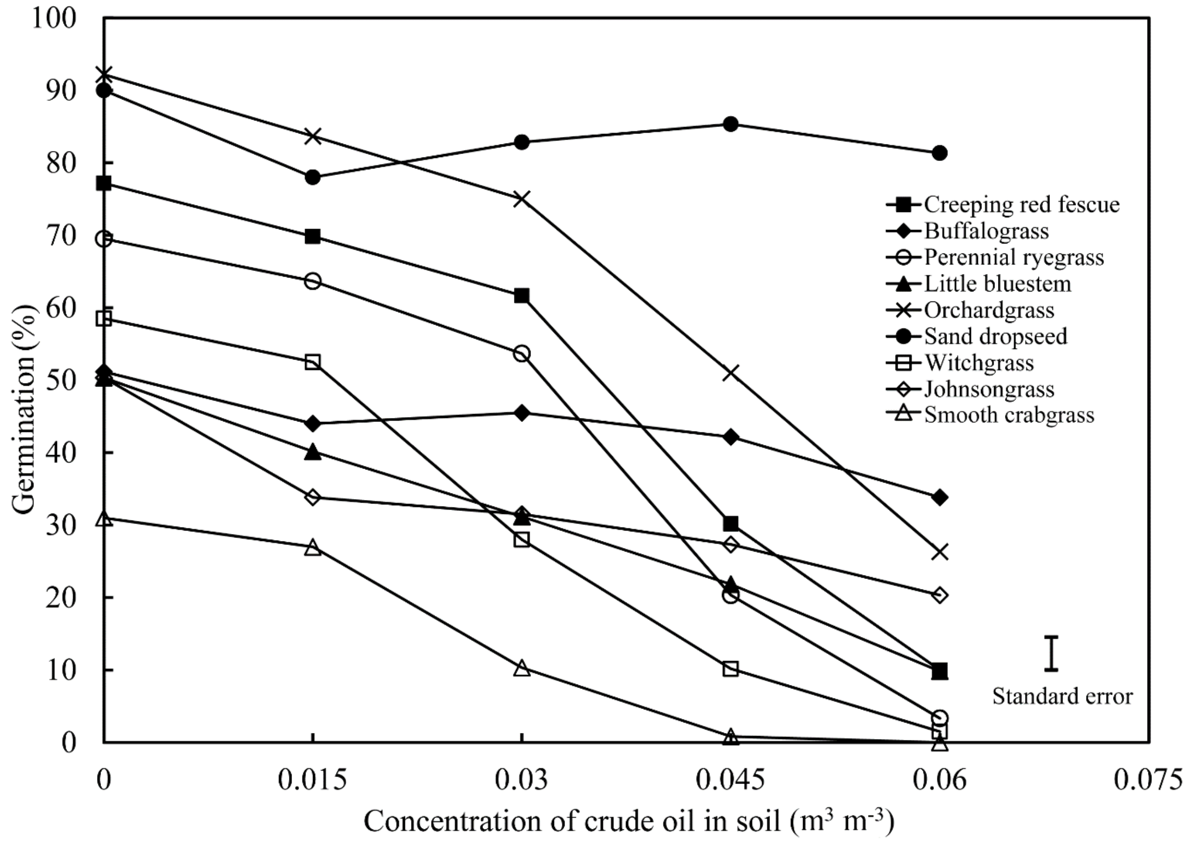

Figure 2.7. Germination of plant species responses to crude oil in the soil.

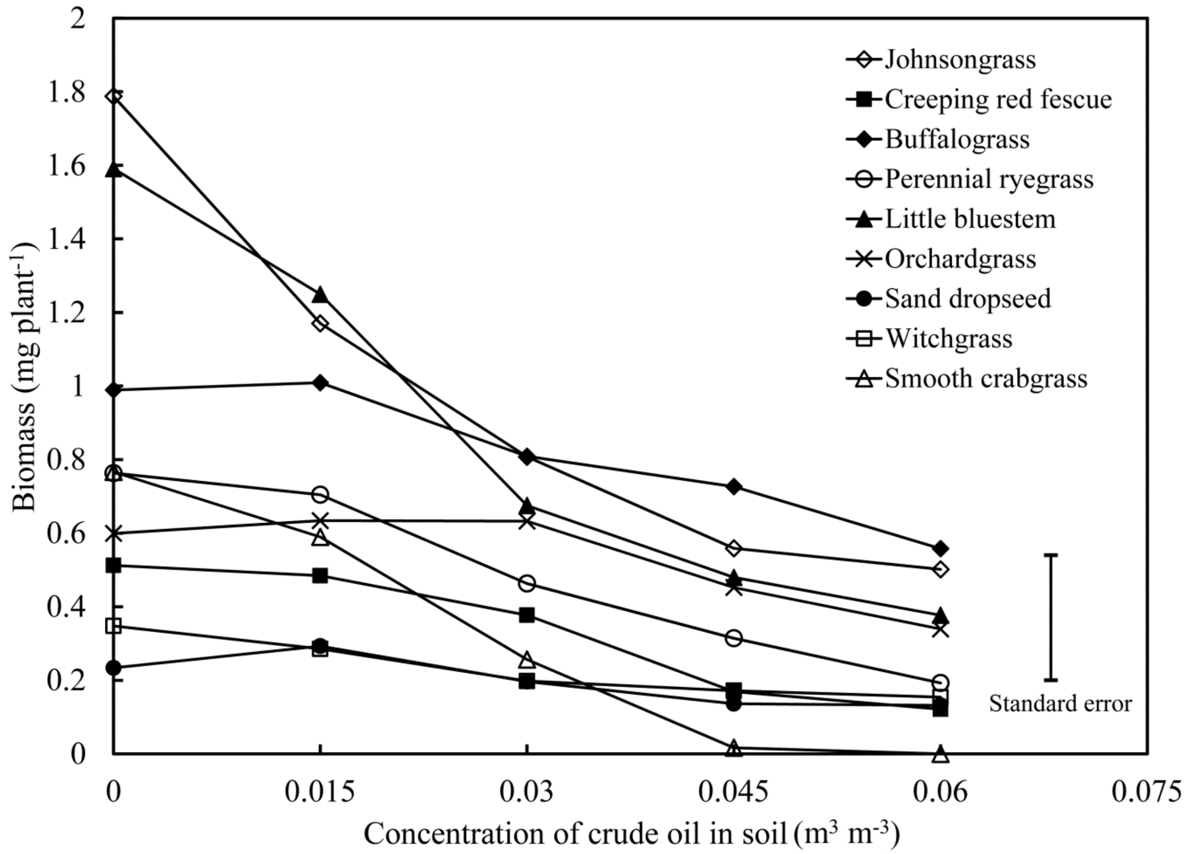

Figure 2.8. Biomass of plant species responses to crude oil in the soil.

Orchardgrass showed great reduction in germination but the final germination and biomass at the highest crude oil concentration were perhaps still high enough to be used in phytoremediation or reclamation of crude oil contaminated soils. However, because of its borderline winter hardiness (Van Santen and Sleper, 1996), its application in North Dakota may be limited. Nevertheless, orchardgrass can be useful for crude oil contamination in soil, which requires sensitivity in germination and sufficient amount of growth for quantification (Banks and Schultz, 2005).

2.4. Conclusions

Germination and seedling biomass of grass species are reduced by drill cuttings and crude oil. There is a large variation between species and genotypes within a species. Comparison of the effect of drill cuttings also depends on sources of drill cuttings which not only affect the TPH content but also pH, salinity, and toxic metals. For phytoremediation and soil reclamation after drill cutting contamination, species with both tolerance to hydrocarbon and salinity is desired. Furthermore, germination and biomass after germination are important factors to select a species for the purpose of phytoremediation. Few grass species met these requirements despite of the large number of grass species evaluated. There was not an observed trend to differentiate native species and introduced species in response to drill cuttings or crude oil. The results found in this study may also help to understand the ecological impact of population shift in a particular location after the contamination or phytoremediation using certain species. For example, downy brome is an invasive weed in North Dakota and it is sensitive to both drill cuttings and crude oil, while witchgrass and yellow foxtail are considered weeds and are tolerant or moderately tolerant to drill cuttings and crude oil.

Salinity adds more stress to seed germination, so many species that are tolerant to crude oil may not be tolerant to drill cuttings. Moderately tolerant species to crude oil were often classified as moderately sensitive or sensitive to drill cuttings. Examples include most cereal crops used in this study. Different responses to drill cuttings and crude oil indicate the mechanisms of inhibition to germination may be different.

Grass seed germination and biomass response to different concentrations of drill cuttings and crude oil further help to explain the possible mechanisms of inhibition and toxicity. Among the possible factors are hydrophobicity, toxic volatile components, salinity, and toxic metals.

However, more research is needed to confirm a specific factor in the role of germination inhibition and toxicity to seedlings. The study also identified species for possible use as indicator plants or bioassay in soil contamination by drill cuttings and crude oil.

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3. GROWTH AND PHYTOTOXICITY OF GRASS SPECIES AFFECTED BY DRILL CUTTINGS AND CRUDE OIL IN SOIL

3.1. Introduction

A successful phytoremediation requires the plants not only to survive the contamination, but to also grow and thrive (Ertekin et al., 2011). More importantly, the reclaimed site should have a sufficient diversity and composition similar to the plant species prior to the contamination in a given location to maintain the ecological functions, such as erosion control, water conservation, and wildlife habitat. Plant species that have good seed germination do not always tolerate drill cuttings or crude oil at seedling or mature stages. Furthermore, different species may have varying ability to reduce hydrocarbons in soil. In a study with sorghum (S*orghum bicolor* L.) and common flax (*Linum usitatissimum* L.), it was found that sorghum germination and subsequent growth was less affected than flax by petroleum hydrocarbon, however, flax showed greater reduction of petroleum hydrocarbons in the contaminated soil in one growing season (Shirdam et al., 2008). Deep-rooted prairie grasses were found effective in reducing the content of polycyclic aromatic hydrocarbons (PAH) in soil (Aprill and Sims, 1990).

Crude oil spills, especially a recent one, can cause injury to plants. Crude oil hydrocarbon toxicity may cause different symptoms. Bermudagrass (*Cynodon dactylon* L.) developed leaf chlorosis and unbranched roots in Maya-crude-oil-polluted soil. The number of leaves, nodes, and branches also decreased (Pena-Castro et al., 2006). The number and width of leaves and stem length of clover (*Trifolium* spp.) were significantly decreased by crude oil contamination in the soil (Ertekin et al., 2011). Freedman and Hutchinson (1976) investigated the effects of crude oil on plant communities and found that vegetative coverage decreased dramatically. *Salix glauca*, *Betula nana*, and *Lupinus arcticus* were discolored and defoliated two weeks after oil spillage, but five weeks later the plants produced regrowth. Lateral buds were stimulated by the crude oil and new leaves with larger surface area developed (Freedman and Hutchinson, 1976).

Hydrocarbons also can cause reduction of chlorophyll content, decrease in nutrient assimilation, and shortening of roots and aerial organs in plants. Anthracene, one of the PAH compounds, inhibits plant photosynthesis by uncoupling phosphorylation (Aksmann et al., 2011). Genotoxicity of nitrobenzene has been detected in tobacco (*Nicotiana tabacum* L.) seedlings by Yuan et al. (2011) at molecular levels.

During oil drilling, drill cuttings are brought to the surface and disposed after recycling the drill mud (drill bit lubricating materials). The composition of drill cuttings is complex and varies from site to site. The well size, drilling material, muds used, environmental conditions, the mineralogy of the strata overlying the target reservoir, and the drilling techniques determine the composition of the drill cutting collectively (Al-Ansary and Al-Tabaa, 2007; Breuer et al., 2004).

Most drill cuttings contain significant amounts of crude oil and may raise the pH (9-12) as well as levels of salts and toxic metals. This may be more detrimental to plant growth than crude oils (Prantera et al., 1991). Significant yield reduction in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) have been reported by Chaineau et al. (1996). Total growth inhibition was recorded for radish (*Raphanus sativus* L.), maize, and barley (*Hordeum vulgare* L.) when drill cuttings were applied at 40 to 50 times the rate of the land spraying while drilling (LWD) for water-based mud system in Alberta, Canada (Saint-Fort and Ashtani, 2014).

Some drill cuttings contain insignificant amounts of crude oil and fewer toxic metals. This type of drill cuttings may even be able to provide extra K, Fe, and Zn to supplement soil fertility and increase crop yield when applied properly (Bauder et al., 1999; Miller and Pesaran, 1980). In these cases, salts were added to soil and the increase in sodium absorption ratio (SAR) of the soil depends on the amount of drill cuttings applied (Bauder et al., 1999). Therefore, whether the application of drill cuttings to agricultural soils is beneficial also depends on soil fertility and other conditions (Saint-fort and Ashtani, 2014). McFarland et al. (1992) transplanted buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) to plots treated with drill fluids and found that canopy cover and above-ground biomass were not affected compared with the untreated control. Also, significant increases in Na, K, and Mg concentrations were found after 17 months of growth in drill fluids-treated plots.

Plant growth and microbial activity may change the hydrocarbon content and chemical properties in drill cuttings or crude oil after plants are introduced (Scelza et al., 2010). Plants may metabolize certain hydrocarbons (Ertekin et al., 2011). Total organic carbon usually decreases with time during phytoremediation (Fan et al., 2014). The distribution in of oil-based hydrocarbons in soil also changes over time (Chaineau et al., 1996).

Oil production has had a very big impact on North Dakota. The state collected $4 billion in oil taxes from July 2011 through June 2013 (Kusnetz, 2014). The oil boom has created new jobs and business opportunities in the state (Kusnetz, 2014). The economic benefit to the state must be balanced against the impact of oil drilling on the environment. Large amounts of drilling waste are generated with oil production. The drilling waste contains high concentrations of hydrocarbons, heavy metals, and salts. Sometimes, drilling waste is radioactive from the shale. The inappropriate disposal or treatment can result in water and soil contamination, also damage vegetation and wildlife. The accidental spill of crude oil or petroleum can also cause detrimental effects because of the high amounts of hydrocarbons (Van Epps, 2006).

A total of 90 native and introduced grass species are found in North Dakota. Grasses are used for forage production, wildlife habitat, conservation, and biofuel production. Some of them have been used to reclaim contaminated soil (Sedivec et al., 2011). Prairie grasses have great potential to be used in the phytoremediation of hydrocarbon-contaminated soil. They have a fibrous root system which results in a large surface area for hydrocarbon-degrading microbes to colonize. Forty-four grass species have been introduced to North Dakota, including Kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* Leyss.), and tall fescue (*Festuca arundinacea* Schreb.). These species can establish quickly because of their competitiveness. However, the effects on mature plants by drill cuttings and crude oil needs to be evaluated. Little is known in the fate of petroleum hydrocarbons after the growth of grass species in the contaminated soil.

The objective of this study was to investigate responses of grass species that are either of high economic value or native ecological importance in North Dakota to drill cuttings and crude oil contamination after germination. The primary goal was to identify species that are tolerant to drill cuttings and/or crude oil contamination for use in phytoremediation or soil reclamation. A secondary objective of this study was to monitor the fate of petroleum hydrocarbon in soil using Fourier transform infrared spectroscopy technology.

3.2. Materials and Methods

3.2.1 Preliminary screening

Seed sources for grass species used in this study are listed in Table 3.1. Seventy-two grass species of high value or natively ecological importance (Table 3.2) were included in this study. The grass seeds were planted in square polyethylene pots measuring 80 mm by 80 mm and 80 mm in depth that were filled with topsoil with a volume of 300 cm^3 per pot. The soil used in this study was a sandy loam (Oye Hubert & Sons Construction, Fargo, ND) with pH of 6.79, electrical conductivity (EC) of 0.235 dS m^{-1}, and bulk density of 1170 kg m^{-3}. The soil was air-dried and sieved to pass a 1-mm screen before use. Prior to start of treatments, the plant materials were maintained in a greenhouse with a 12-h photoperiod and supplementary lights from metal halide light bulbs. Watering was conducted daily and a liquid fertilizer 9N-18P_2O_5-9K_2O was applied weekly at 12 mL L^{-1} (fertilizer/water).

Table 3.1. Sources of seeds used in the preliminary screening for tolerance to drill cutting and crude oil contamination at the mature stage.

Company/Facility name	City	State	Abbreviations
Agassiz Seed & Supply	Fargo	ND	AGS
Aberdeen PMC†	Aberdeen	ID	ABD
Bismarck PMC	Bismarck	ND	BSM
Bridger PMC	Bridger	MT	BRD
Elstel Farm & Seed	Thomas	OK	EFS
Jacklin Seed Co.	Post Falls	ID	JKL
Knox City PMC	Knox City	TX	KXC
Lockeford PMC	Lockeford	CA	LKF
Los Lunas PMC	Los Lunas	NM	LLN
Millborn Seeds	Brookings	SD	MLB
North Dakota State University	Fargo	ND	NDSU
Prairie Restoration Inc.	Princeton	MN	PRR
Pullman PMC	Pullman	WA	PLM
Rivard's TURF & FORAGE	Grand Forks	ND	RWD
SIMPLOT Jacklin seed division	Post Falls	ID	SPL
Tee-2-Green Corp.	Hubbard	OR	TTG
Twin City Seed Co.	Edina	MN	TCS
University of Minnesota	Minneapolis	MN	UOM
Upper Colorado Environmental Plant Center	Meeker	CO	UCEPC
Williams Lawn Seed	Maryville	MO	WLS

† Plant material center.

Table 3.2. Plant species used for the preliminary screening for tolerance to drill cuttings and crude oil contamination in soil at the mature plant stage.

Common name	Scientific name	Variety	Seed source†	Common name	Scientific name	Variety	Seed source
Kentucky bluegrass	*Poa pratensis* L.	Park	AGS	RS hybrid wheatgrass	*Elymus hoffmannii* Jensen & Asay	Saltlander	RWD
Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	Red River	EFS	Intermediate wheatgrass	*Agropyron intermedium* (Host.) Beauv.	Manifest	BSM
Creeping bentgrass	*Agrostis stolonifera* L.	Penn 4	TTG	Big bluestem	*Andropogon gerardii* Vitman	Bison	RWD
Colonial bentgrass	*Agrostis capillaris* L.	Alister	TTG	Western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Löve	Rodan	BSM
Red top	*Agrostis gigantea* L.	VNS‡	WLS	Russian wildrye	*Elymus junceus* Fisch.	Mankota	BSM
Strong creeping red fescue	*Festuca rubra* L. ssp. *rubra*	Navigator II	AGS	Desert wheatgrass	*Agropyron desertorum* (Fisch. ex Link) Schult.	Nordan	BSM
Rough bluegrass	*Poa trivialis* L.	Laser	TCS	Siberian wheatgrass	*Agropyron fragile* (Roth) Candargy	Vavilov II	ABD
Chewings fescue	*Festuca rubra* L. ssp. *Commutata* (Thuill.)	Intrigue	TCS	Bluebunch wheatgrass	*Pseudoroegneria spicata* (Pursh) A. Löve	Anatone	ABD
Buffalograss	*Buchloe dactyloides* (Nutt.) Engelm.	Bowie	RWD	Beardless wheatgrass	*Pseudoroegneria spicata* (Pursh) Love ssp. *inermis* (Scribn & Sm.) A. Löve	Whitmar	PLM
Perennial ryegrass	*Lolium perenne* L.	Panther	RWD	Pubescent Intermediate wheatgrass	*Agropyron trichophorum*	Manska	MLB
Hard fescue	*Festuca trachyphylla* (Hackel) Krajina	Firefly	TCS	Sand bluestem	*Andropogon hallii* Hack.	Elida	LLS

(continues)

Table 3.2. Plant species used for the preliminary screening for tolerance to drill cuttings and crude oil contamination in soil at the mature plant stage. (continued)

Common name	Scientific name	Variety	Seed source†	Common name	Scientific name	Variety	Seed source
Kentucky bluegrass	*Poa pratensis* L.	Bewitched	TCS	Thickspike wheatgrass	*Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould	Sodar	MLB
Hybrid crested wheatgrass	*Agropyron desertorum* (Fisch. ex Link) J.A. Schultes ×*Agropyron cristatum* (L.) Gaertn.	HyCrest	UCEP	Canada bluegrass	*Poa compressa* L.	Cannon	MLB
Sheep fescue	*Festuca ovina* L.	Blue Ray	AGS	Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	'Bad lands' ecotype	BSM
Annual ryegrass	*Lolium multiflorum* Lam.	VNS	AGS	Maize	*Zea mays* L.	NDBS1011	NDSU
Slender wheatgrass	*Elymus trachycaulus* (Link) Gould ex Shinners	Revenue	RWD	Hard red spring wheat	*Triticum aestivum* L.	Glenn	NDSU
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	Itasca	RWD	Hard red winter wheat	*Triticum aestivum* L.	Jerry	NDSU
Weeping alkaligrass	*Puccinellia distans* (Jacq.) Parl.	Fults	RWD	Oat	*Avena sativa* L.	Jury	NDSU
Timothy	*Phleum pratensis* L.	Climax	RWD	Durum wheat	*Triticum durum* L.	Tioga	NDSU
Tall wheatgrass	*Agropyron elongatum* (Host.) Beauv.	Alkar	RWD	Sweet corn	*Zea mays* L. var. *saccharata*	Synergy	NDSU
Orchardgrass	*Dactylis glomerata* L.	Potomac	RWD	Beardless wildrye	*Leymus triticoides (Buckl.) Pilg.*		
Meadow brome	*Bromus biebersteinii* Roem.	Fleet	RWD	Tall fescue	*Festuca arundinacea* Schreb.	RIO	LKF

(continues)

Table 3.2. Plant species used for the preliminary screening for tolerance to drill cuttings and crude oil contamination in soil at the mature plant stage. (continued)

Common name	Scientific name	Variety	Seed source†	Common name	Scientific name	Variety	Seed source
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	Pierre	RWD	Tufted hairgrass	*Deschampsia caespitosa P. Beauv*	Shade	UOM
Canada wildrye	*Elymus Canadensis* L.	Mandan	RWD	Idaho bentgrass	*Agrostis idahoensis* Nash	Golfstar	JKL
Virginia wildrye	*Elymus virginicus* L.	MN native	RWD	Annual bluegrass	*Poa annua L.*	VNS	UOM
Canada bluegrass	*Poa compressa* L.	Foothills	BRD	Foxtail barley	*Hordeum jubatum* L.	VNS	NDSU
Creeping meadow foxtail	*Alopecurus arundinaceus* Poir.	Garrison	BRD	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	VNS	NDSU
Basin wildrye	*Leymus cinereus* (Scribn. & Merr.) Löve	Trailhead	BRD	Johnsongrass	*Sorghum halepense* (L.) Pers.		NDSU
Prairie sandreed	*Calamovilfa longifolia* (Hook.) Scribn.	Goshen	BRD	Japanese brome	*Bromus japonicus* Thunb.	VNS	NDSU
Thickspike wheatgrass	*Elymus lanceolatus* (Scribn. & J.G.Sm.) Gould	Critana	BRD	Quackgrass	*Elymus repens* (L.) Gould	VNS	NDSU
Sand bluestem	*Andropogon hallii* Hack.	Chet	MLB	Downy brome	*Bromus tectorum* L.	VNS	NDSU
Fairway crested wheatgrass	*Agropyron cristatum* (L.) Gaertn.	Douglas	ABD	Barnyardgrass	*Echinochloa crus-galli* (L.) P. Beauv.	VNS	NDSU
Mammoth wildrye	*Leymus racemosus* (Lam.) Tzvelev	Volga	PLM	Smooth crabgrass	*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.	VNS	NDSU

(continues)

Table 3.2. Plant species used for the preliminary screening for tolerance to drill cuttings and crude oil contamination in soil at the mature plant stage. (continued)

Common name	Scientific name	Variety	Seed source†	Common name	Scientific name	Variety	Seed source
Switchgrass	*Panicum virgatum* L.	Forestburg	RWD	American sloughgrass	*Beckmannia syzigachne* (Steud.) Fern.	VNS	PRR
Green needlegrass	*Nassella viridula* (Trin.) Barkworth	Lodorm	RWD	Fowl bluegrass	*Poa palustris* L.	VNS	PRR
Indiangrass	*Sorghastrum nutans* (L.) Nash	Tomahawk	RWD	Barley	*Hordeum vulgare* L.	Pinnacle	NDSU

† Seed source refers to Table 3.1.

‡Variety not stated (VNS).

3.2.1.1. Drill cuttings effect

Oil drill cuttings (Pioneer Energy Services Corp., San Antonio, TX) from the Bakken oil field in western North Dakota, had a sodium absorption ratio (SAR) of 47.7, EC of 5.0 dS m^{-1}, pH 9.8, and total petroleum hydrocarbon (TPH) 108,100 mg kg^{-1}, and Ca, Mg, Mn, Na, Cl, and HCO_3 were 502, 1150, 3.5, 8460, 6820, and 1810 mg kg^{-1}, respectively. The Fourier Transform Infrared (FTIR) spectrum of the drill cuttings is shown in Fig. 3.1.

Drill cutting contaminated soil was prepared by mixing top soil with drill cutting at 1:2 (V/V) ratio. The contaminated soils were kept in a 24-L plastic container outdoors for 10 d to

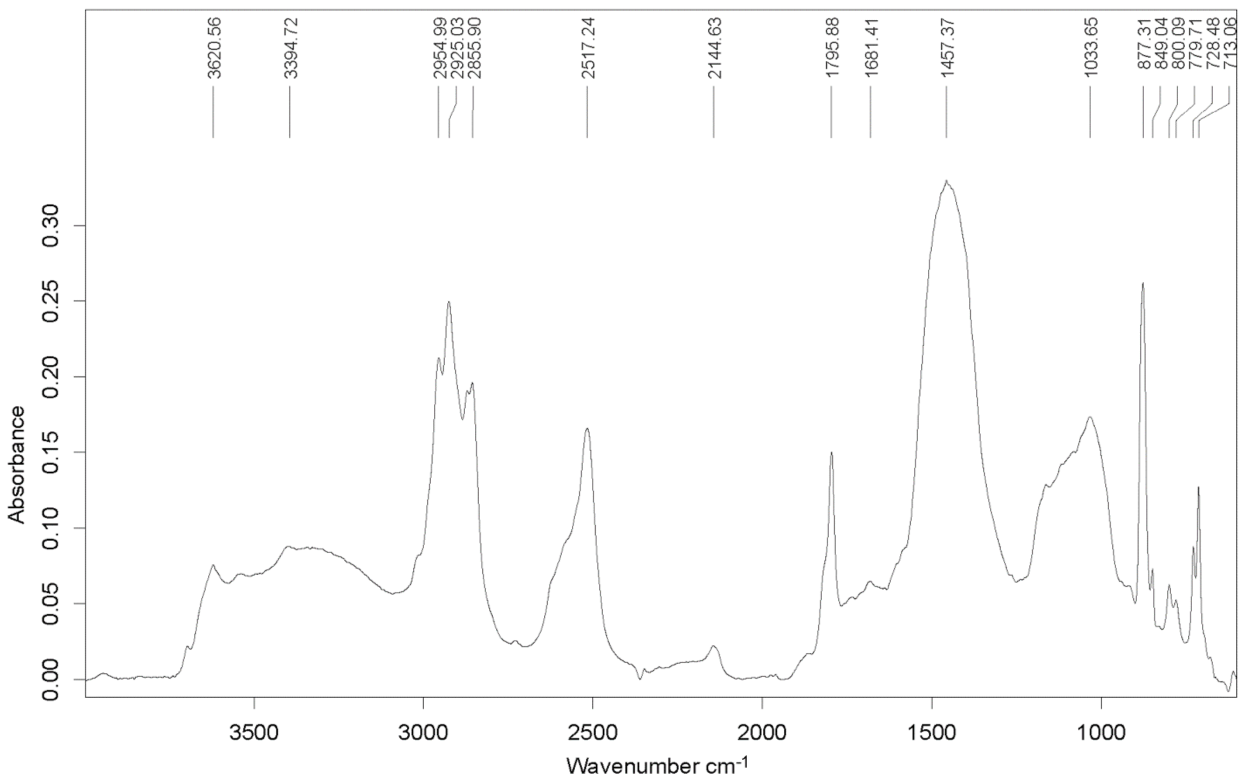

Figure 3.1. Absorbance spectrum of drill cuttings in the soil from Bakken oil fields in western North Dakota.

alleviate odor prior to sealed storage in a 4°C walk-in cooler during the experiment. The vegetative stage was defined as when first tiller appeared. This stage occurred as early as 5-leaf stage (Hyder, 1974; Langer, 1956; Skinner and Nelson, 1994). Once the one of the 72 species reached the 5-leaf stage, 80 cm^3 contaminated soil was added into the pot and mixed with a screw driver to the original soil to reach the final contamination concentration of 1:10 (V/V). A 0.5-cm layer of charcoal was spread on the surface of contaminated soils to absorb odors and to prevent fumes from contaminating the greenhouse air. Greenhouse photoperiod and supplemented lights were described in the previous section. The treatments were arranged in a randomized complete block design with three replicates. The experiment was conducted once.

3.2.1.2. Crude oil effect

Crude oil (Tesoro Refining & Marketing Co. San Antonio, TX) from Bakken oil fields in western North Dakota used in this study contained a total petroleum hydrocarbon (TPH) of 99%, among which were 1% N-hexane, 1.5% benzene, 0.1% naphthalene, and 0.1% xylene. The FTIR spectrum of the crude oil is shown in Fig. 3.2. The top soil was spiked with crude oil at 1:10 (V/V), which was equivalent to a spill of 5 L crude oil on 1 m^2 of soil to a depth of 15 cm. The contaminated soils were kept in a 24-L plastic container outdoors to alleviate odors prior to storage in a walk-in cooler at 4°C for this experiment. Once the 72 species reached the 5-leaf stage, 80 cm^3 of contaminated soil was added to the top of the pot containing the grass species and tilled in with a screw driver to make the final concentration of contamination to 0.021 m^3 m^{-3} (V/V). Other treatment application procedures were as mentioned above. Untreated species were used as control. The treatments were arranged in a randomized complete block design with three replicates. The experiment was conducted once.

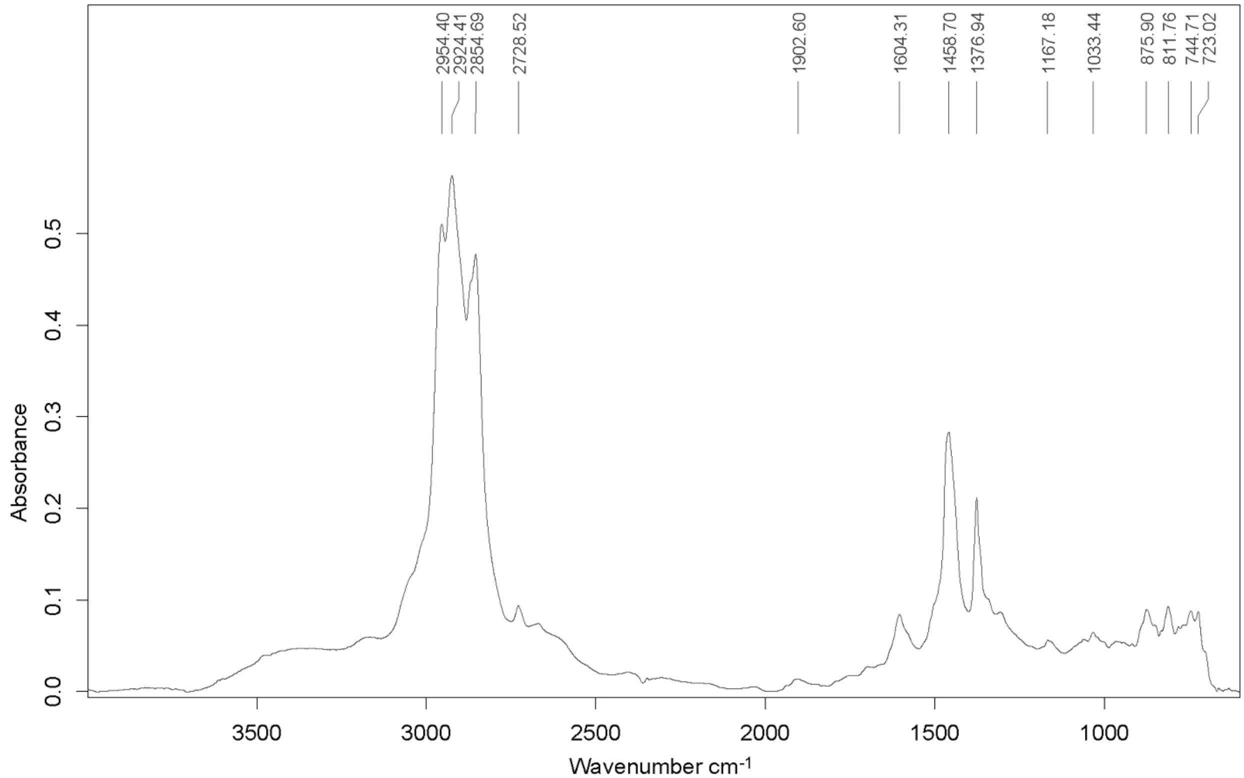

Figure 3.2. Absorbance spectrum of crude oil in the soil from Bakken oil fields in western North Dakota.

3.2.1.3. Measurements and data analysis

The plants were watered daily using an automatic irrigation system to prevent water stress. No fertilizer was applied to avoid the interaction of fertilizer with the drill cuttings or crude oil.

Plant injury was visually evaluated 4 weeks after the treatment using an evaluating system with a scale from 0 to 100, where 0 means no crop reduction or injury and 100 indicates complete crop destruction (Camper, 1986). Plant biomass above the soil surface was harvested 6 weeks after the treatment and oven-dried at 80°C for 24 h to determine the dry weight. Plant biomass reduction as compared with the untreated control also were calculated.

Plant visual injury, biomass, and biomass reduction data were tested with capability procedures in SAS for the normality of distribution and analyzed using ANOVA and the general linear model in SAS (SAS Institute, 2013). Mean separation was done using F-protected Tukey test at 0.05 significance level. Replicates were considered as random effects and treatments and species were considered as fixed effects.

3.2.2. Growth and phytotoxicity of grass species at different levels of contamination

Nine species were selected based on the results of the preliminary screening to represent plants with different levels of tolerance to drill cuttings and crude oil. These species were 'Pinnacle' barley, yellow foxtail (variety not stated) (VNS), quackgrass (VNS), 'Laser' rough bluegrass, 'Revenue' slender wheatgrass, annual ryegrass (VNS), 'Bowie' buffalograss, 'Vavilov II' Siberian wheatgrass, and 'Whitmar' beardless wheatgrass. Seeds of these species were planted in 72-cell germination flats at 2 to 3 seeds per cell. The flats were maintained in a greenhouse under automatic mist irrigation and 12-h photoperiod with supplementary lights from metal halide light bulbs. Seedlings were thinned to one per cell after germination and were fertilized with liquid fertilizer 9N-18P_2O_5-9K_2O at 12 mL L^{-1} (fertilizer /water) weekly.

3.2.2.1. Drill cuttings dose effect

The same top soil used in the preliminary screening was used for this study. It was sieved to pass a 1-mm screen before use. To create different levels of contaminations, drill cuttings were mixed thoroughly with soil at concentrations of 0, 0.05, 0.10, 0.15, and 0.20 m^3 m^{-3} on volumetric bases. All mixtures were prepared one day before use. Each square pot measured 80 mm × 80 mm × 80 mm was filled with about 350 mL contaminated soil or untreated soil.

At the 5-leaf stage, plants from germination flats were carefully removed. After the soil was carefully washed off the root system with tap water, three plants from the same species were transplanted into each pot filled with the contaminated soil. Plants transplanted to pots with uncontaminated soil were used as control. The treatments were arranged in randomized complete block design with three replicates. The plants were watered immediately after transplanting and maintained in a greenhouse with supplementary lights from metal halide light bulbs. The environmental conditions were recorded using a Watchdog mini weather station (Spectrum technologies, Inc. Aurora, IL.). The experiment was repeated once.

3.2.2.2. Crude oil dose effect

To prepare for crude oil contaminated soil, crude oil was mixed with top soil at rates of 0, 0.015, 0.030, 0.045, and 0.064 $m^3\ m^{-3}$ one day before the use. When a species reached the 5-leaf stage, they were transplanted to crude oil contaminated soil as described above for drill cuttings treatment with uncontaminated soil as control. The treatments were arranged as randomized complete block design with three replicates. The experiment was repeated once.

3.2.2.3. Measurement and data analysis

Soil samples were taken from the mixtures of different concentrations of drill cuttings or crude oil and soil for the tests of pH and EC. The soil pH was tested in a 1:1 soil/ deionized water (V/V) suspension using a multi-parameter meter (HQ40d, Hach Company, Loveland, CO) and the EC was determined in a 1:5 soil/deionized water (V/V) extract using an EC meter (model 1054, VWR Scientific, Radnor, PA).

Soil samples also were taken from each pot 4 weeks after treatment (WAT) using a 5-mm diameter soil probe and air-dried under a ventilation hood at room temperatures. The soil samples were ground with an agate mortar and pestle to fine particles passing through a 100-mesh sieve. A 0.01 g subsample was then weighed out from each soil sample using an analytical balance and mixed with 0.09 g KBr for dilution. The mixture was then further ground to powder using a small agate mortar and pestle to obtain the samples that were qualified for the FTIR analysis. The samples were loaded into a diffuse reflectance sample holder and scanned at a resolution of $4 cm^{-1}$ with 64 scans in the mid infrared (MIR) range of 4000-600 cm^{-1} (Tensor 27, Bruker Optics Inc., Billerica, MA). Absorbance peaks of the FTIR spectrum were identified for different functional groups and tested for their prediction ability for the main component of crude oil, and alkane. Standard curves were established from the samples with known concentrations of drill cuttings and crude oil using regression procedures in SAS (SAS institute, 2013) with y as absorbance and x as concentration of contaminants. The order of polynomial regression was decided by the significance of the coefficient at 0.05 level of probability.

The spectra were baseline corrected and absorbance peaks were identified using OPUS software (Bruker Optics Inc., Billerica, MA). The peaks then were assigned to different chemical groups for different basic vibrations and overtones (Coates, 2000; Lv et al., 2012). The standard curves were used to predict residue chemical groups in the soils after growing different species for 4 weeks. Soil samples from controls that had no grass planted were used as base line to calculate the reduction of crude oil in soil samples that had different levels of contamination and different species grown in them.

Visual injury of plants from drill cuttings and crude oil were evaluated using a 0 to 100 scale as in the preliminary experiment (Camper, 1986). At the time of injury evaluation, a photo was taken for each pot as a record. At the end of study, the biomass above soil surface from each pot was harvested and rinsed with tap water to get rid of the soil attached to plants. The plant material was oven-dried at 80°C for 24 h to determine the dry weight.

Data were tested with capability procedures in SAS for the normality of distribution. The data were subjected to ANOVA using general linear model in SAS with experiment and block as random variables, and species and concentrations of crude oil were fixed effects. Mean separation was done with F-protected Tukey test at 0.05 significance level.

3.3. Results and Discussion

3.3.1. Preliminary screening

The data of phytotoxicity as indicated by visual rating were normally distributed. ANOVA for phytotoxicity (Table 3.3) showed that there was a significant difference among species treated with drill cuttings and crude oil, and. Significant difference in the biomass, and biomass reduction as compared with untreated control also existed (Tables 3.4 and 3.5). The significance in block effects indicated that the blocking was effective and variation within the block was reduced.

Table 3.3. ANOVA of visual rating of mature plants caused by drill cuttings and crude oil in the soil for the preliminary screening.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Block	2	67.6	0.6	0.5476	266.5	3.7	0.0268
Species (S)	71	2022.5	18.1	<0.0001	2172.2	30.3	<0.0001
Error	142	111.8			71.8		
Total	215						

Table 3.4. ANOVA of biomass of mature plants affected by drill cuttings and crude oil in the soil for the preliminary screening.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Block	2	0.30	3.8	0.0245	1.32	15.9	<0.0001
Species (S)	71	5.00	62.3	<0.0001	4.22	50.8	<0.0001
Treatment (T)	1	29.92	372.9	<0.0001	43.79	526.8	<0.0001
S × T	71	0.20	2.4	<0.0001	0.31	3.7	<0.0001
Error	286	0.08			0.08		
Total	431						

Table 3.5. ANOVA of biomass reduction of mature plants affected by drill cuttings and crude oil in the soil for the preliminary screening.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr > F	MS	F	Pr > F
Block	2	961.3	2.93	0.0564	6885.4	23.4	<0.0001
Species (S)	71	828.4	2.53	<0.0001	788.9	2.7	<0.0001
Error	142	327.6			294.4		
Total	215						

3.3.1.1. Grass growth in soil contaminated with drill cuttings

Mean visual injuries and biomass reduction caused by drill cuttings compared with the untreated control for the 72 grass species are shown in Table 3.6. Fourteen species, among which seven are cereal crops, showed visual injury index less than 20. Chaineau et al. (1996) also reported that maize and wheat were successfully cultivated and harvested with drill cuttings treatment, but showed significant yield reduction compared with the untreated control. Eleven species, of which six were wheatgrass species, had visual injury higher than 80 and major biomass reduction. However, visual injury was not always accompanied by proportional biomass reduction because visual evaluation of phytotoxicity included stunting of growth, abnormal morphology, chlorosis, and loss of stands (Camper, 1986).

Of the grass species screened, grassy weeds ranked in the top one-third of biomass reduction with only yellow foxtail, foxtail barley, and Johnsongrass as exceptions (Table 3.6). These grassy weeds were quackgrass, Japanese brome, downy brome, meadow brome, large crabgrass, and barnyardgrass. Although yellow foxtail and Johnsongrass had biomass reduction more than 50%, it had normal seed production. The moderate tolerance of grassy weeds to drill cuttings indicated that if drill cuttings are disposed on existing vegetation, those weeds may be selectively retained and result in major damage to the ecological balance of the grassland.

Table 3.6. Visual rating (VR), biomass, and biomass reduction (Red.) of grass species affected by drill cuttings (DC) in the soil.

Species	VR	Biomass			Species	VR	Biomass		
		Control	DC	Red.			Control	DC	Red.
		—g pot^{-1}—		–%–			—g pot^{-1}—		–%–
Barley	3.3	2.27	2.11	6.9	Large crabgrass	58.3	1.72	1.35	21.5
Hard red spring wheat	3.3	1.69	1.47	13.0	Pubescent Intermediate wheatgrass	58.3	1.53	0.96	37.0
Maize	5.0	4.97	3.36	32.4	Tall fescue	58.3	2.43	1.89	22.0
Yellow foxtail	5.0	2.35	1.15	51.1	Chewings fescue	60.0	1.39	1.14	17.9
Hard red winter wheat	8.3	2.63	1.64	37.4	Canada bluegrass	60.0	1.25	0.60	52.0
Durum wheat	8.3	1.67	1.30	22.0	American sloughgrass	60.0	0.96	0.79	17.4
Quackgrass	8.3	1.79	1.23	31.6	Red top	61.7	0.81	0.83	14.7
Orchardgrass	10.0	2.65	1.46	45.1	Tall wheatgrass	61.7	1.99	1.39	30.2
Oat	10.0	1.88	1.60	14.9	Western wheatgrass	61.7	1.48	1.34	9.5
Japanese brome	10.0	1.52	1.16	26.2	Tufted hairgrass	61.7	2.47	1.72	30.4
Downy brome	10.0	1.25	0.87	29.9	Colonial bentgrass	63.3	0.60	0.48	20.0
Rough bluegrass	11.7	1.67	1.01	39.5	Beardless wildrye	63.3	1.77	1.01	42.9
Sweet corn	11.7	7.64	6.45	15.6	Foxtail barley	63.3	1.96	1.17	40.5
Annual bluegrass	16.7	2.53	1.57	37.9	Sand bluestem	65.0	3.58	2.56	28.4
Mammoth wildrye	33.3	2.12	1.24	41.7	Johnsongrass	66.7	1.74	0.73	57.8
Hybrid crested wheatgrass	35.0	1.01	0.40	60.3	Desert wheatgrass	68.3	0.95	0.47	50.1
Creeping meadow foxtail	35.0	1.04	0.87	16.3	Intermediate wheatgrass	71.7	1.30	1.05	19.1
Hard fescue	40.0	1.78	1.63	8.5	Sand bluestem	73.3	2.55	1.40	45.2
Kentucky bluegrass†var.1	43.3	1.91	1.31	31.4	Indiangrass	75.0	1.21	1.09	9.8
Russian wildrye	43.3	1.03	0.57	44.6	Creeping bentgrass	78.3	1.80	1.22	32.2
Little bluestem‡var.1	45.0	1.15	0.97	15.7	Basin wildrye	78.3	1.15	0.76	34.2
Perennial ryegrass	46.7	1.38	1.07	22.0	Prairie sandreed	78.3	2.06	1.79	13.1
Canada bluegrass	46.7	0.78	0.29	62.8	Sheep fescue	80.0	2.09	1.96	6.2
Slender wheatgrass	48.3	1.51	1.06	29.8	Weeping alkaligrass	80.0	2.07	1.69	18.4
Little bluestem‡var.2	48.3	2.03	1.21	40.4	Barnyardgrass	80.0	0.30	0.29	4.7
Idaho bentgrass	48.3	1.46	0.47	67.6	Canada wildrye	81.7	1.30	0.88	32.0
Strong creeping red fescue	50.0	2.42	1.48	39.0	Kentucky bluegrass†var.2	85.0	1.52	1.08	29.0
Annual ryegrass	50.0	1.70	1.17	31.4	Buffalograss	85.0	1.73	0.78	55.2

(continues)

Table 3.6. Visual rating (VR), biomass, and biomass reduction (Red.) of grass species affected by drill cuttings (DC) in the soil. (continued)

Species	VR	Biomass			Species	VR	Biomass		
		Control	DC	Red.			Control	DC	Red.
		—g pot^{-1}—		–%–			—g pot^{-1}—		–%–
RS hybrid wheatgrass	50.0	1.56	1.01	35.1	Virginia wildrye	85.0	1.20	1.02	15.3
Big bluestem	51.7	1.07	0.83	22.7	Siberian wheatgrass	85.0	1.22	0.60	51.1
Switchgrass	53.3	1.08	0.85	21.3	Sideoats grama	86.7	2.03	0.91	55.4
Smooth crabgrass	53.3	1.98	1.25	37.1	Bluebunch wheatgrass	86.7	0.47	0.11	77.7
Fowl bluegrass	53.3	1.54	0.76	50.6	Thickspike wheatgrass	88.3	2.15	1.15	46.6
Green needlegrass	55.0	0.96	0.73	24.2	Fairway crested wheatgrass	91.7	0.55	0.20	63.6
Timothy	56.7	1.20	1.04	13.3	Thickspike wheatgrass	95.0	1.33	0.76	43.0
Meadow brome	56.7	1.70	1.16	32.0	Beardless wheatgrass	96.7	0.69	0.22	68.0
$HSD_{0.05}$§	17.1	0.32	0.32	29.2	$HSD_{0.05}$	17.1	0.32	0.32	29.2

†Kentucky bluegrass var.1 is ‘Bewitched’ and Kentucky bluegrass var.2 is ‘Park’.
‡Little bluestem var.1 is ‘Itasca’ and Little bluestem var.2 is ‘Bad Land’ ecotype.
§Tukey’s Studentized Range (HSD) at the 0.05 probability level.

Variation existed within a genus and there was not a trend of drill cutting tolerance for the 12 genera that included more than two species in the study. The *Triticum* genus was the only one consistently tolerant to drill cuttings among species. There were also variations in drill cuttings tolerance among varieties of Kentucky bluegrass, which is an introduced species and considered invasive in forage crops (Grant et al., 2009). Kentucky bluegrass has been artificially selected with many commercial varieties (NTEP, 2014). On the other hand, native little bluestem has less variation within the species.

Although the TPH content in the drill cuttings used in this study was comparable with many other reports ranging from 4.2 to 22.4% (W/W) (Al-Ansary and Al-Tabbaa, 2007; Breuer et al., 2004), direct comparison between these results and previous studies is complicated because of the different hydrocarbon chemical components and salt content (Anoliefo et al., 2006). The drill cuttings used in this study had a high SAR and relatively high EC, indicating that an effect of salinity on the growth of these grasses also needs to be considered.

<u>3.3.1.2. Grass growth in soil contaminated with crude oil</u>

Similar to the responses to drill cuttings, cereal crops used in this study were more tolerant to crude oil than most of other grass species (Table 3.7). Thirty nine species had visual rating less than 20, while 14 species were in this range when grown in soil contaminated with drill cuttings. Since the drill cuttings had high pH and salt content in addition to significant amount of crude oil, it is possible that the different responses to drill cuttings and oil were due to salinity and alkalinity sensitivities. For example, slender wheatgrass was ranked very sensitive to salinity stress (Dewey, 1960), and the visual injury rate and biomass reduction were increased from 10 and 27.8% to 48.3 and 29.8% for crude oil and drill cuttings treatments, respectively (Tables 3.6 and 3.7).

Table 3.7. Visual rating (VR), biomass, and biomass reduction (Red.) of grass species affected by crude oil (Oil) in the soil.

Species	VR	Biomass			species	VR	Biomass		
		Control	Oil	Red.			Control	Oil	Red.
		—g pot^{-1}—		–%–			—g pot^{-1}—		–%–
Barley	0.0	2.27	2.24	1.2	Timothy	20.0	1.20	0.88	26.8
Hard red spring wheat	0.0	1.69	1.20	28.9	Meadow brome	20.0	1.70	1.19	30.1
Sweet corn	0.0	7.64	5.32	30.4	Russian wildrye	20.0	1.03	0.72	30.6
Yellow foxtail	0.0	2.35	0.95	59.5	Large crabgrass	21.7	1.72	1.11	35.4
Creeping meadow foxtail	3.3	1.04	0.77	26.0	Sand bluestem	21.7	2.55	1.16	54.7
Oat	3.3	1.88	1.42	24.5	Orchardgrass	23.3	2.65	1.54	42.1
Hard red winter wheat	5.0	2.63	1.40	46.5	Indiangrass	26.7	1.21	0.97	19.8
Durum wheat	6.7	1.67	1.16	30.5	Sand bluestem	26.7	3.58	1.85	48.3
Quackgrass	6.7	1.79	1.19	33.5	Hard fescue	30.0	1.78	1.25	29.7
Downy brome	6.7	1.25	0.92	26.2	Kentucky bluegrass‡var.1	30.0	1.91	1.14	40.3
Annual ryegrass	8.3	1.70	1.28	24.8	Prairie sandreed	30.0	2.06	1.40	32.1
Rough bluegrass	10.0	1.67	0.99	41.0	Strong creeping red fescue	33.3	2.42	1.55	36.0
Chewings fescue	10.0	1.39	1.01	27.3	Tufted hairgrass	33.3	2.47	1.20	51.3
Slender wheatgrass	10.0	1.51	1.09	27.8	Perennial ryegrass	36.7	1.38	1.09	21.1
Little bluestem†var.1	10.0	1.15	0.88	23.5	Little bluestem†var.2	36.7	2.03	1.16	42.9
Maize	10.0	4.97	3.02	39.3	Fowl bluegrass	36.7	1.54	0.68	55.6
Annual bluegrass	10.0	2.53	1.65	34.9	Pubescent Intermediate wheatgrass	40.0	1.53	1.11	27.5
Japanese brome	10.0	1.52	1.25	17.8	Canada bluegrass	41.7	0.78	0.45	42.5
Colonial bentgrass	11.7	0.60	0.12	80.5	Foxtail barley	46.7	1.96	1.14	41.5
Big bluestem	11.7	1.07	0.73	31.8	Beardless wildrye	50.0	1.77	1.15	35.0
Western wheatgrass	11.7	1.48	1.13	23.2	Idaho bentgrass	55.0	1.46	0.54	62.7
Red top	13.3	0.95	0.63	34.2	Desert wheatgrass	60.0	0.95	0.61	35.1
Tall wheatgrass	13.3	1.99	1.47	26.4	Weeping alkaligrass	63.3	2.07	1.46	29.4
Canada bluegrass	13.3	1.25	0.53	57.9	Buffalograss	66.7	1.73	0.60	65.3
Mammoth wildrye	13.3	2.12	1.51	28.7	Intermediate wheatgrass	66.7	1.30	1.02	21.5
Green needlegrass	13.3	0.96	0.74	22.9	Canada wildrye	68.3	1.31	0.72	44.7
Johnsongrass	13.3	1.74	0.46	73.9	Kentucky bluegrass‡var.2	73.3	1.52	0.78	48.9
Barnyardgrass	13.3	0.30	0.24	21.8	Creeping bentgrass	75.0	1.80	0.99	44.9

(continues)

Table 3.7. Visual rating (VR), biomass, and biomass reduction (Red.) of grass species affected by crude oil (Oil) in the soil. (continued)

Species	VR	Biomass			species	VR	Biomass		
		Control	Oil	Red.			Control	Oil	Red.
		—g pot^{-1}—		–%–			—g pot^{-1}—		–%–
Hybrid crested wheatgrass	15.0	1.01	0.44	56.3	Sideoats grama	76.7	2.03	0.84	58.8
Smooth crabgrass	15.0	1.98	0.66	66.5	Basin wildrye	78.3	1.15	0.77	32.9
American sloughgrass	15.0	0.96	0.76	21.3	Virginia wildrye	80.0	1.20	0.81	32.0
Sheep fescue	16.7	2.09	1.54	26.3	Bluebunch wheatgrass	85.0	0.47	0.10	78.8
Thickspike wheatgrass	16.7	2.15	1.08	49.9	Siberian wheatgrass	88.3	1.22	0.66	45.7
Switchgrass	18.3	1.08	0.99	8.0	Thickspike wheatgrass	88.3	1.33	0.96	27.7
RS hybrid wheatgrass	18.3	1.56	0.88	43.7	Fairway crested wheatgrass	91.7	0.55	0.20	64.4
Tall fescue	18.3	2.43	1.89	22.3	Beardless wheatgrass	91.7	0.69	0.13	81.3
$HSD_{0.05}$§	27.7	0.33	0.33	13.7	$HSD_{0.05}$	27.7	0.33	0.33	13.7

†Little bluestem var.1 is ‘Itasca’ and Little bluestem var.2 is ‘Bad Land’ ecotype.

‡Kentucky bluegrass var.1 is ‘Bewitched’ and Kentucky bluegrass var.2 is ‘Park’.

§Tukey’s Studentized Range (HSD) at the 0.05 probability level.

Beardless wheatgrass was ranked most sensitive among 25 *Agropyron* species (Dewey, 1960), and also most sensitive to drill cuttings and crude oil in this study.

There were variations in crude oil tolerance among varieties within a species and among species within a genus. Different concentration levels of the contaminants will be needed to further test the tolerance of a species. Grassy weeds such as quackgrass, Japanese brome, downy brome, large crabgrass, and barnyardgrass showed to be very tolerant or moderately tolerant to crude oil based on the visual rating and biomass reduction. Therefore, the ecological impacts of crude oil on the species composition of natural grassland need to be evaluated where those species exist. On the other hand, native species 'Forestburg' switchgrass and 'Itasca' little bluestem showed to be very tolerant to crude oil contamination. Species showing different tolerance levels as indicated by both different germination and various degree of visual injury levels were included for further evaluation of the response to different concentrations of contaminants in soil (Table 3.8).

Table 3.8. Visual rating (VR), biomass and biomass reduction (Red.) of grass species from the screening study used for the dose effect of both drill cuttings (DC) and crude oil (Oil) on mature plants.

Species	VR	Biomass			VR	Biomass		
		Control	DC	Red.		Control	Oil	Red.
		—g pot^{-1}—		%		—g pot^{-1}—		%
Barley	3.3	2.27	2.12	6.6	0.0	2.27	2.25	0.9
Yellow foxtail	5.0	2.35	1.15	51.1	0.0	2.35	0.95	59.6
Quackgrass	8.3	1.79	1.23	31.3	6.7	1.79	1.20	33.0
Rough bluegeass	11.7	1.67	1.01	39.5	10.0	1.67	0.99	40.7
Slender wheatgrass	48.3	1.51	1.06	29.8	10.0	1.51	1.09	27.8
Annual ryegrass	50.0	1.70	1.17	31.2	8.3	1.70	1.28	24.8
Buffalograss	85.0	1.73	0.77	55.5	66.7	1.73	0.60	65.3
Siberian wheatgrass	85.0	1.22	0.60	50.8	88.3	1.22	0.66	45.9
Beardless wheatgrass	96.7	0.69	0.22	68.1	91.7	0.69	0.13	81.2

3.3.2. Growth and phytotoxicity of grass species affected by different concentrations of drill cuttings and crude oil in the soil

3.3.2.1. Drill cuttings dose effect

The data of phytotoxicity as indicated by visual rating and plant biomass were normally distributed. Both plant biomass and phytotoxicity levels were affected by levels of drill cuttings in soil (Table 3.9). Grass species did not showed significant differences, but significant interaction with experiment and concentration levels was detected. Therefore, grass performance is presented by each experiment.

Table 3.9. ANOVA of the biomass and visual rating of grass species affected by drill cuttings in the soil.

		Biomass			Visual rating		
Source of variation	df	MS	F	Pr>F	MS	F	Pr>F
Exp	1	0.935	8.2	0.021	15855	5.3	0.0443
Block within Exp	4	0.019	1.6	0.184	65	1.7	0.1459
Species (S)	8	0.149	2.4	0.124	1839	0.9	0.5625
Concentration (C)	4	4.973	79.1	0.001	71667	60.0	0.0008
S × C	32	0.017	0.9	0.665	234	0.9	0.6311
Exp × S	8	0.063	3.3	0.007	2062	7.8	<0.0001
Exp× C	4	0.063	3.3	0.024	1191	4.5	0.0052
Exp × S × C	32	0.019	1.6	0.033	263	7.0	<0.0001
Error	176	0.012			38		

Visual rating of phytotoxicity showed the levels of injury increased with increasing concentrations of drill cuttings in the soil (Fig. 3.3). The grass biomass decreased with increasing concentrations of drill cuttings (Fig. 3.4). Grasses ranked differently in Experiment (Exp.) 1 and Exp. 2, especially by the visual ratings. This was likely caused by the different environmental conditions (Fig. 3.5). Relative humidity was lower in Exp. 1 than Exp. 2, and more extreme temperatures occurred during Exp. 1. Biomass reduction as affected by drill cuttings also showed different results between Exp. 1 and Exp. 2 (Fig. 3.4), but in both experiments, yellow foxtail and barley showed greater biomass values at the end of study than other species. Different results in each experiment indicate that grass development stage, temperature, relative humidity, and PAR light also needs to be included in further studies when comparing grass species of different growth habits (Anslow and Green, 1967; Fleming and Murphy, 1968). Also, plant response to contaminants during vegetative and reproductive growth need to be further evaluated. In this study, yellow foxtail showed injury by drill cuttings but was successful in seed production.

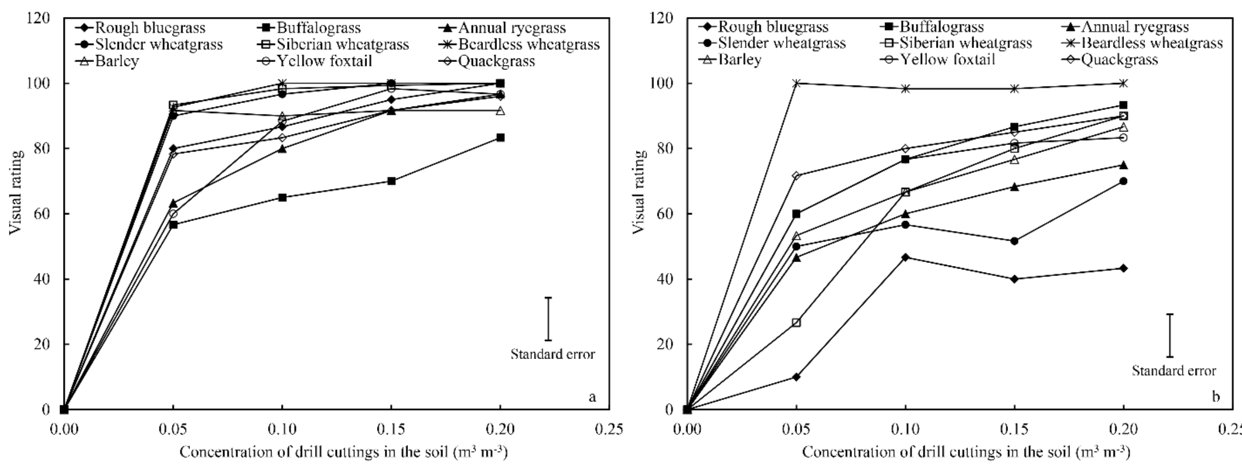

Figure 3.3. Visual rating of selected plant species affected by drill cuttings in the soil (a is the Experiment 1 and b is the Experiment 2).

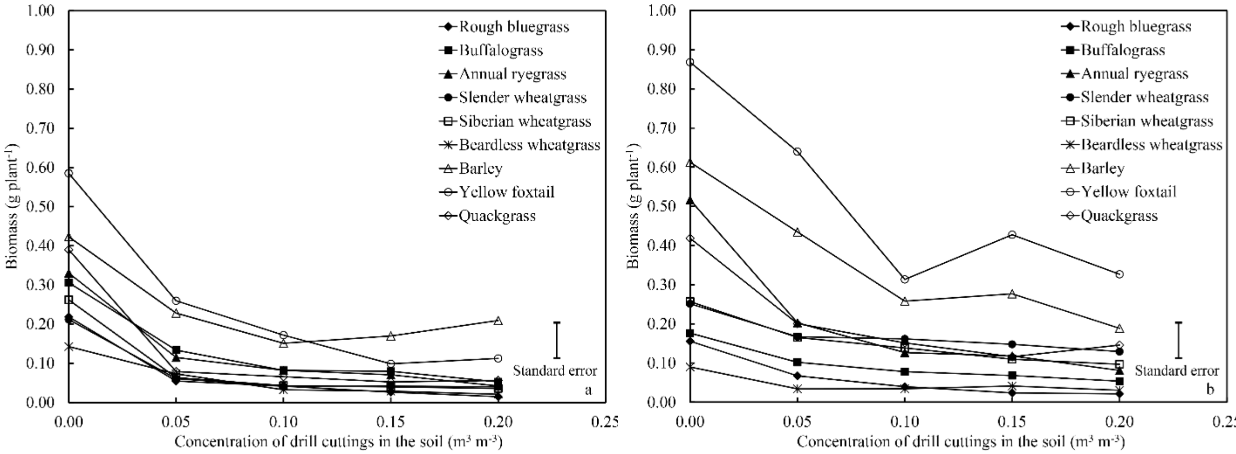

Figure 3.4. Biomass of selected plant species affected by drill cuttings in the soil (a is Experiment 1, and b is Experiment 2).

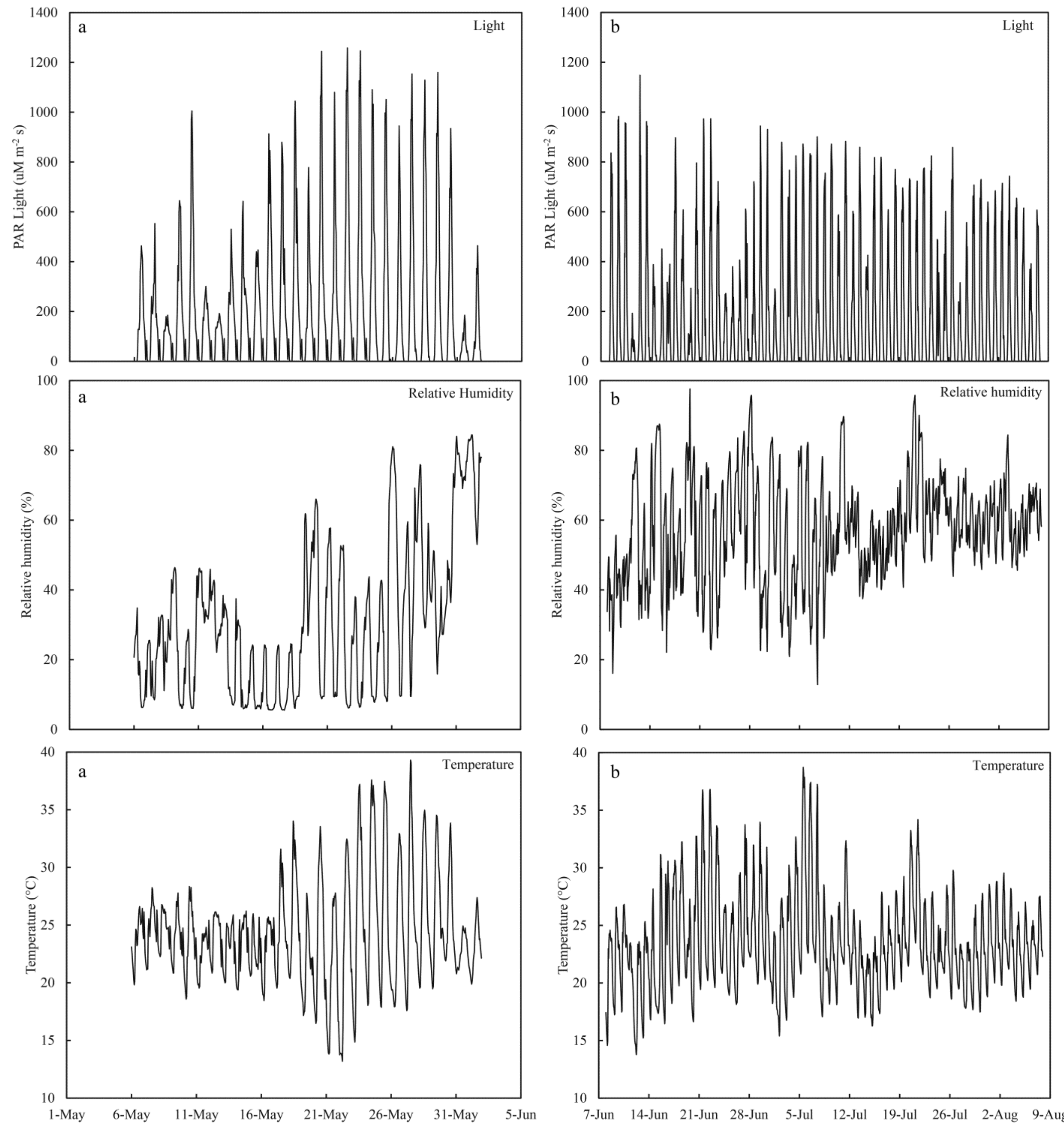

Figure 3.5. Photosynthetically active radiant (PAR), relative humidity, and temperature during the study period (a is Experiment 1, and b is Experiment 2).

The salinity levels of drill cuttings contaminated soil is shown in Figure 3.6. As the concentration of drill cuttings in the soil increased, the salinity levels of the contaminated soil increased. Therefore, the plant responses to drill cuttings may be contributed by salinity. The results were in agreement with the salinity

tolerance levels reported for barley, slender wheatgrass and Siberian wheatgrass (Beauchamp, 2009; Dewey, 1960; Katerji et al., 2009).

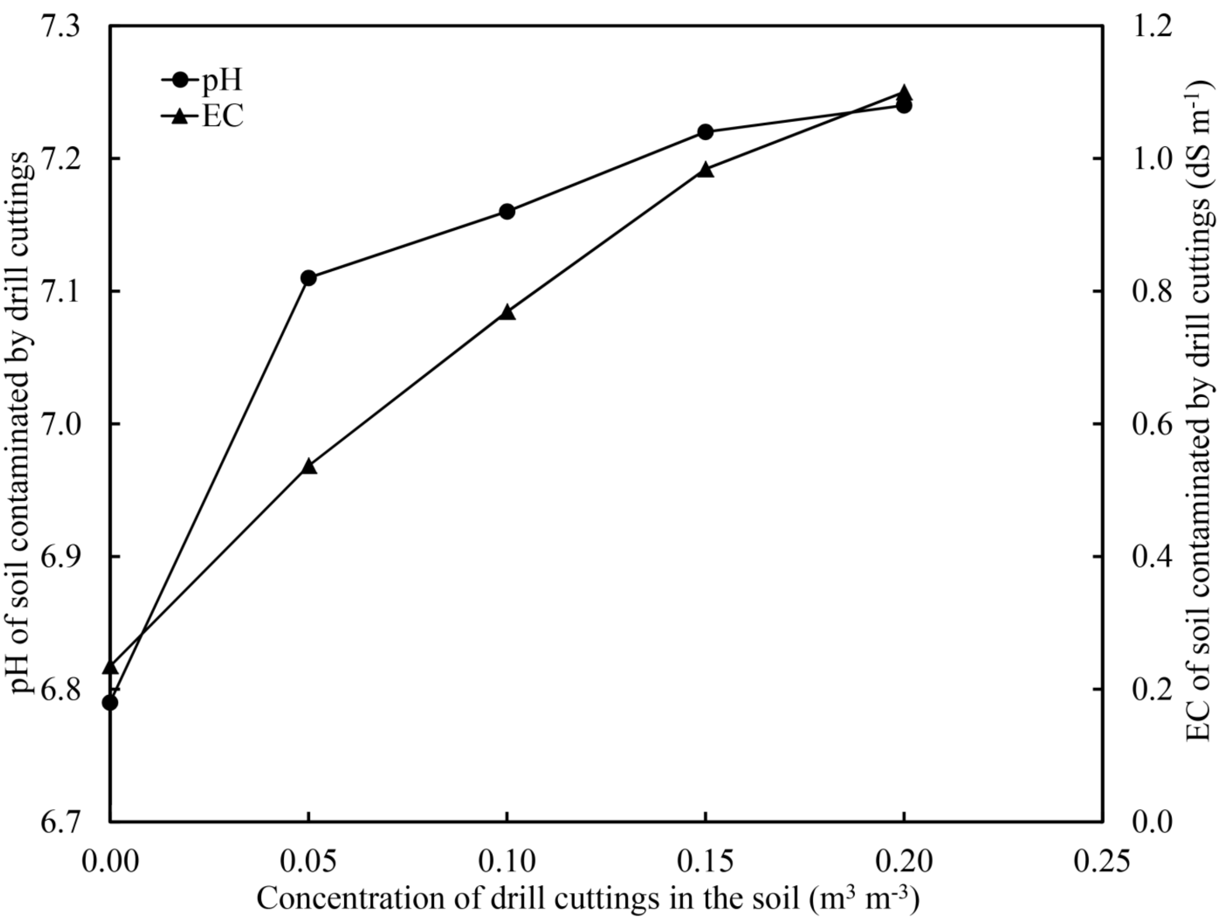

Figure 3.6. pH and electrical conductivity (EC) of the soil contaminated by drill cuttings.

3.3.2.2. Crude oil dose effect

Biomass and phytotoxicity were affected by different levels of crude oil contaminations in soil (Table 3.10). The species effect was not significant but interaction with experiment and concentration were significant. Therefore, results are presented by each experiment.

Table 3.10. ANOVA of the biomass and visual rating of grass species affected by crude oil in the soil.

		Biomass			Visual rating		
Source of variation	df	MS	F	Pr>F	MS	F	Pr>F
Exp	1	0.92	4.2	0.072	10742	1.8	0.2152
Block within Exp	4	0.07	3.5	0.009	195	2.3	0.0570
Species (S)	8	0.22	2.5	0.107	3081	0.6	0.7411
Concentration (C)	4	2.72	22.9	0.005	51526	30.7	0.0029
S × C	32	0.04	1.1	0.401	346	0.6	0.9460
Exp × S	8	0.09	2.5	0.032	4948	8.0	<0.0001
Exp× C	4	0.12	3.4	0.021	1680	2.7	0.0462
Exp × S × C	32	0.04	1.9	0.005	616	7.4	<0.0001
Error	176	0.02			83		

Both biomass reduction and phytotoxicity ratings increased with increasing concentrations of crude oil in the soil (Figs. 3.7 and 3.8). The trends were different between Exp. 1 and Exp. 2, especially for the visual ratings. This may be because of different environmental conditions during the two experiments (Fig. 3.5). The sensitivity of grass growth and development to environmental may have affected the results.

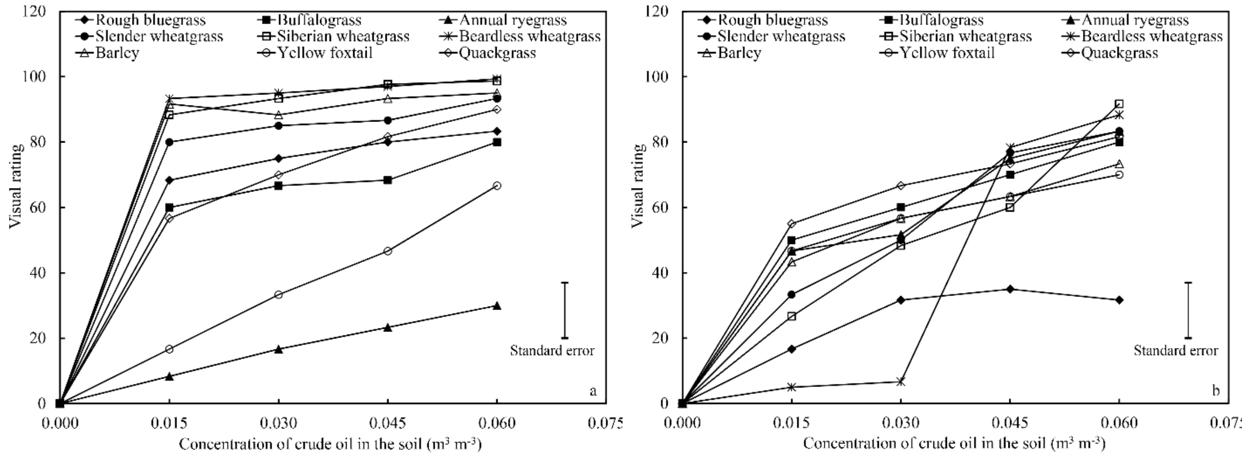

Figure 3.7. Visual rating of plant species affected by crude oil in the soil (a is Experiment 1, and b is Experiment 2).

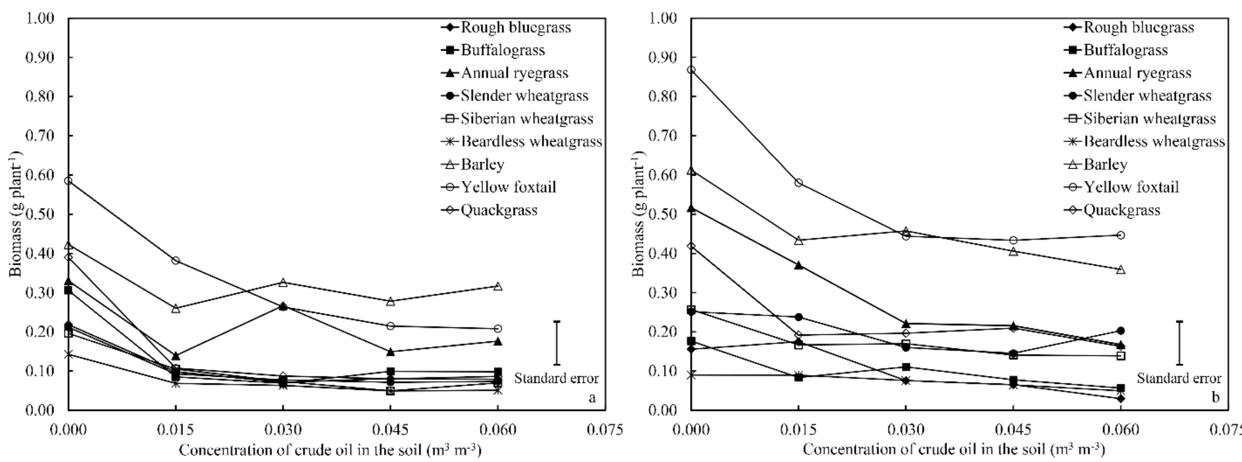

Figure 3.8. Biomass of plant species affected by crude oil in the soil (a is Experiment 1, and b is Experiment 2).

Nevertheless, yellow foxtail and barley showed less biomass reduction and relatively high biomass at the end of study in both experiments. As in the case of drill cuttings treatment, barley and yellow foxtail were able to produce seeds despite of the visual injury. These two species representing C3 and C4 types and maybe used for further study on the mechanisms of their responses to petroleum hydrocarbon because hydrocarbon has been reported to adversely affect photosynthesis by affecting electron transport in photosystem I (Huang et al., 1997).

Soil pH and EC did not significantly change as oil concentration increased (Fig. 3.9). The responses from the grass species in this study were contributed to hydrocarbons. Our results for cereal crops tolerance are in agreement with other studies (Paskova et al., 2006), where different components of hydrocarbons have been reported affecting grasses differently (Kang et al., 2010). Additional research is needed to study grass tolerance to different components of crude oil.

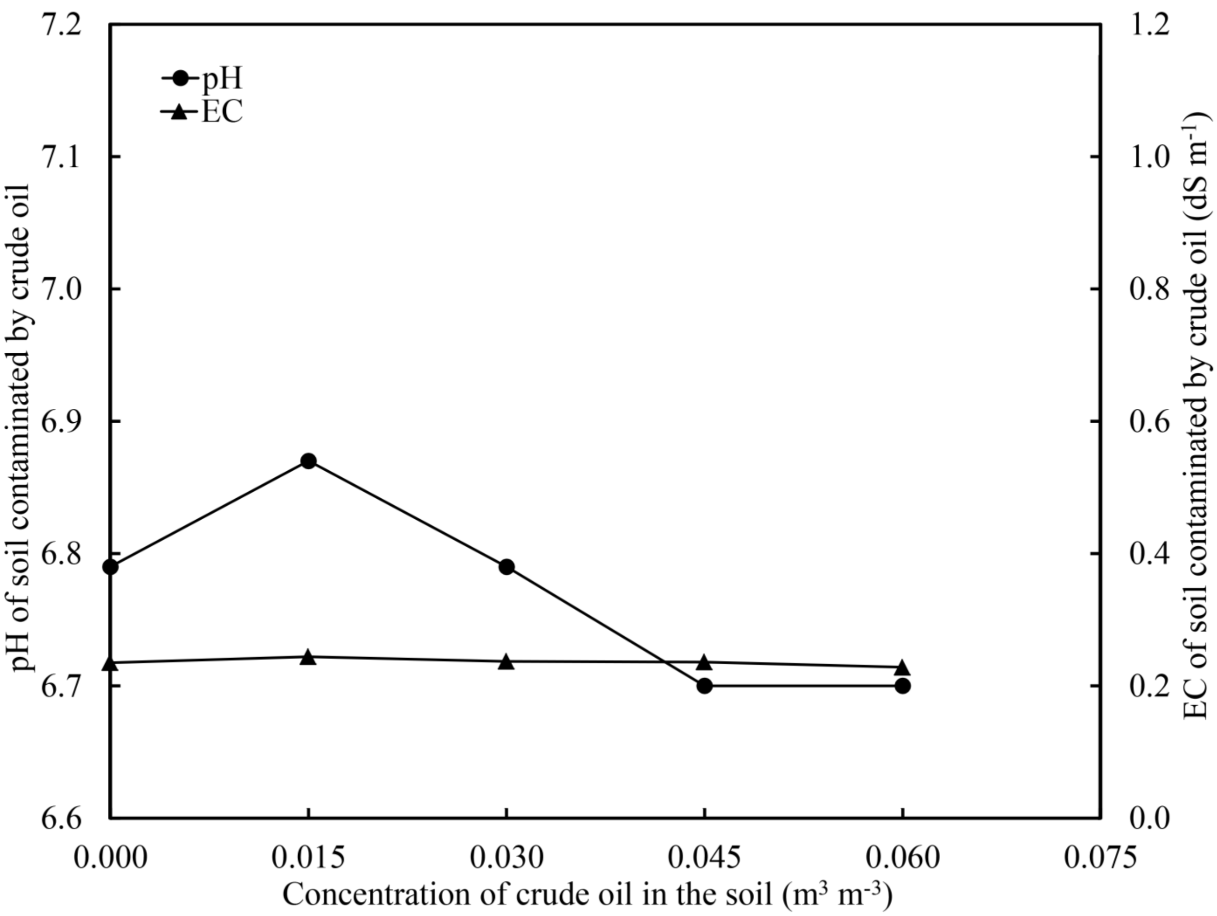

Figure 3.9. pH and electrical conductivity (EC) of the soil contaminated by crude oil.

3.3.2.3. Prediction of residue contaminants in soil from FT-MIR spectroscopy

The absorbance spectra for FTIR showed typical peaks for different function groups that are typical for crude oil and drill cuttings (Table 3.11). Typical FTIR spectra are shown in Figs. 3.1 and 3.2. Therefore, alkanes, alkenes, aromatic components, could be qualitatively and quantitatively measured using this technology. Alkyl halide and carbonyl may also be included in the drill cuttings and crude oils based on the absorbance peaks at wavenumber 2728, 1167, and 812 cm^{-1}. Those components are highly toxic to environment as carcinogens (Aksmann et al., 2011; Baker, 1970).

Absorbance peak at wavenumber 2925 cm^{-1} was found strongly indicative of both drill cuttings and crude oil concentration in soil. The absorbance response to various concentrations of drill cuttings and crude oil were shown in fig 3.10 and 3.11, respectively. The regression equations were

$$Y=0.05 + 0.31x - 0.14x^2,\ r^2=0.99 \qquad (1)$$

$$Y=0.08 + 1.88x - 2.97x^2,\ r^2=0.98 \qquad (2)$$

for drill cuttings and crude oil, respectively. Y is absorbance, and x is concentration of drill cuttings and crude oil, respectively.

Using equation 1 as prediction model, the crude oil residues from drill cuttings contamination in soil after 4 weeks of growth of grass species were predicted and the levels were significantly affected by both species and concentrations of crude oil and by species only for drill cuttings (Table 3.12). The species main factor difference indicated that different species were able to reduce the crude oil components in drill cuttings differently, and the ability was not dependent on levels of contaminations tested (Table 3.13).

Table 3.11. Assignment of peaks in Fourier Transform Infrared (FTIR) absorbance for drill cuttings and crude oil contaminated soil.

Wavenumber (cm^{-1})	Function groups	Vibration
2954	Alkane, C-H	Stretch†
2925	Alkane, C-H	Stretch
2854	Alkane, C-H	Stretch
2728	Aldehyde	Stretch
2517		Overtone‡ of 1459 cm^{-1} and 1033 cm^{-1}
2144	Alkynes, -C≡C-	Stretch
1902	Benzene ring	Overtone
1795		Overtone of 1033 cm^{-1} and 713 cm^{-1}
1681	-C=O	Stretch
1604	C-C (in-ring)	Stretch
1459	Carbonyl, -C=O	Stretch
1376	Alkane, C-H	Rock§
1167	Alkyl halide, $-CH_2X$	Wag¶
1033	Aromatics, C-H	Stretch
877	C-O	In-plane bending#
875	C-O	In-plane bending
849	C-O	In-plane bending in vaterite and aragonite
812	Alkyl halides, C-Cl	Stretch
745	Alkene, =C-H	Bending
728	Alkane, C-H	Rock
723	Alkane, C-H	Rock
713	C-O	Out-plane bending†† in calcite

† Stretch means a change in the length of a bond.
‡ Overtone means an intense peak will display a smaller peak at a multiple of that peak.
§ Rock means a change in angle between a group of atoms.
¶ Wag means a change in angle between the plane of a group of atoms.
# In-plane bending means a change in the angle between two bonds in the same plane.
†† Out-plane bending means a change in the angle between any one of the C-H bonds and the plane defined by the remaining atoms of the ethylene molecule.

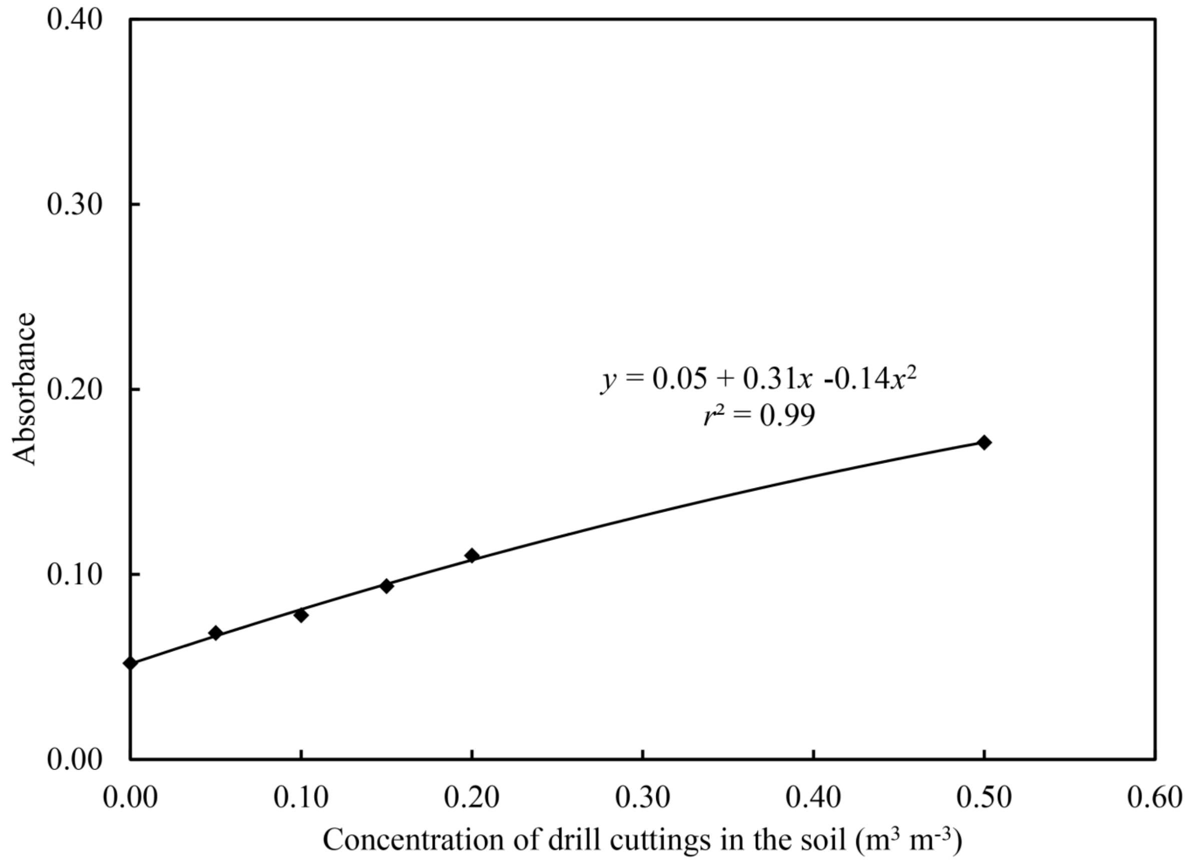

Figure 3.10. Absorbance of soil contaminated by drill cuttings at different concentrations at $2925 cm^{-1}$.

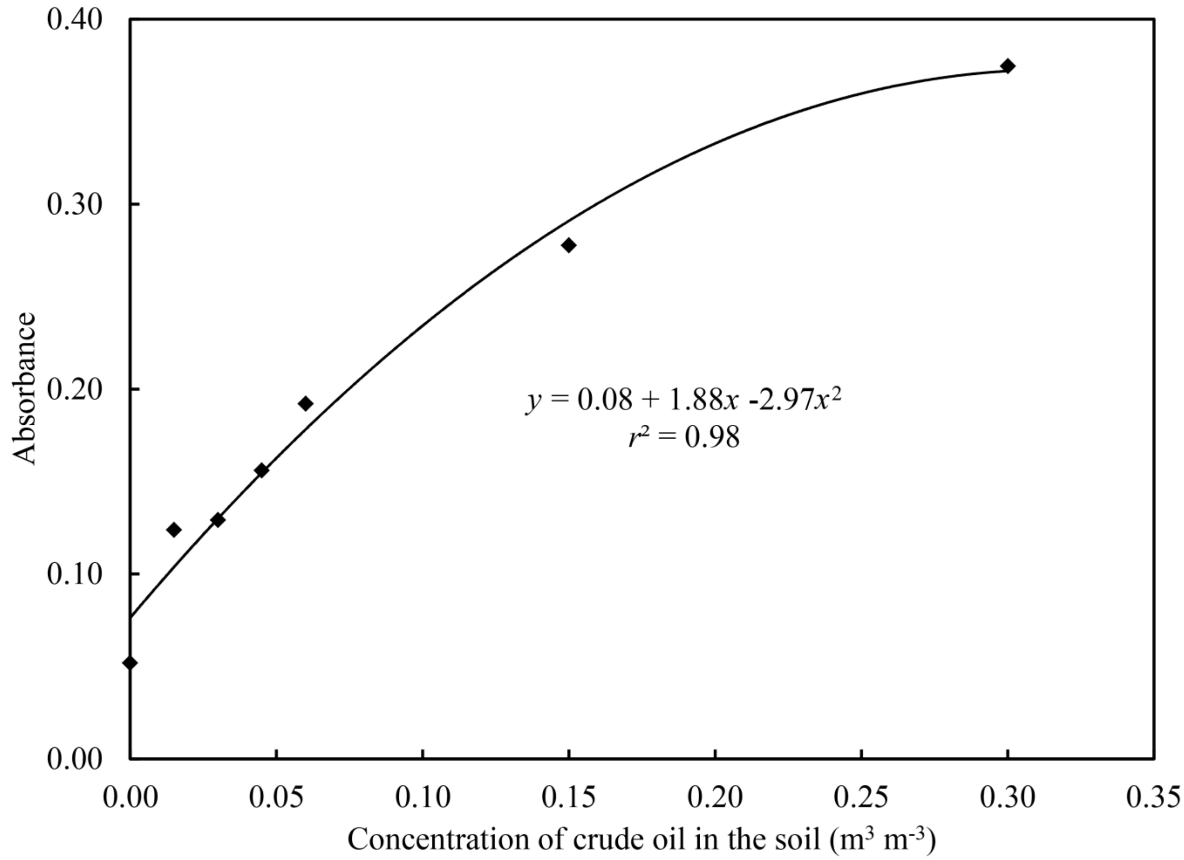

Figure 3.11. Absorbance of soil contaminated by drill cuttings at different concentrations at 2925cm^{-1}.

Table 3.12. ANOVA of the concentration of total hydrocarbon in the soil contaminated by drill cuttings and crude oil affected by selected plant species by using Fourier Transformed Infrared (FTIR) absorbance spectra at 2925 cm^{-1}.

		Drill cuttings			Crude oil		
Source of variation	df	MS	F	Pr>F	MS	F	Pr>F
Rep	2	0.0009	2.6	0.0842	0.000023	3.2	0.0438
Species (S)	8	0.0106	7.9	<0.0001	0.000073	10.1	<0.0001
Concentration (C)	4	0.0006	0.9	0.4735	0.000059	8.2	<0.0001
S × C	32	0.0038	0.7	0.8602	0.000011	1.5	0.0630
Error	88	0.0002			0.000007		
Total	134						

Table 3.13. The concentration reduction of total hydrocarbon in the soil contaminated by drill cuttings and crude oil affected by selected plant species by using Fourier Transformed Infrared (FTIR) absorbance spectra at 2925 cm^{-1}.

Species	Drill cuttings	Crude oil
	$m^3 m^{-3}$	
Annual ryegrass	0.0028	0.0042
Barley	0.0026	0.0037
Yellow foxtail	0.0013	0.0061
Quackgrass	0.0013	0.0044
Rough bluegrass	0.0009	0.0002
Beardless wheatgrass	0.0007	0.0009
Siberian wheatgrass	0.0003	0.0010
Slender wheatgrass	0.0008	0.0008
Buffalograss	0.0001	0.0002
$LSD_{0.05}$	0.0010	0.0019

Grass species also showed difference in the ability of decrease hydrocarbon in soil (Table 3.13). Annual ryegrass and barley showed higher ability than other species for removing hydrocarbons from soil contaminated either by crude oil or drill cuttings.

Similarly, carbonates, cyclopropane derivative or azide residues from drill cuttings contamination also can be predicted using absorbance peaks at 877 and 1033 cm^{-1} respectively. However, since the actual content of those components were not tested in drill cuttings and crude oil, their absolute amount will not be discussed here. Further study is needed to evaluate the dynamics of those components during the phytoremediation processes using grass species.

Despite of the growth tolerance of buffalograss in the soil contaminated by drill cuttings or crude oil, it showed the lowest removal of hydrocarbons from the soil, along with Siberian wheatgrass and slender wheatgrass. All of these results indicated that different tolerance mechanisms may exist and require more study.

3.4. Conclusions

Grass species that are important in North Dakota showed different levels of tolerance to drill cuttings contamination and crude oil contaminations. The phytotoxicity levels may be different from the biomass production under contamination conditions. For remediation purposes, tolerant species can be used; while for reclamation purposes, species composition that existed in the area prior to contamination has to be restored. Therefore, transplanting of established species that are tolerant to contaminations should be used in combination with direct seeding for the phytoremediation and reclamation purposes.

Native species showed different levels of tolerance compared to introduced species and weeds, therefore, ecological impact caused by oil spill and/or drill cuttings contamination need to be evaluated on the contaminated sites.

Different grass species also demonstrated different levels of remediation capabilities in terms of reducing the contaminants in the soil. FTIR spectroscopy can be used not only to identify the levels of contaminant residues in the soil but also to identify the derivatives of the contaminants.

3.5. References

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4. GENERAL DISCUSSION AND CONCLUSION

4.1. The Role of Grasses in Phytoremediation

Grass species make up a large percentage of the natural habitat and crops in the Bakken oil and gas production areas of western North Dakota. Al grass species are of great importance as forages, in soil and water conservation, wildlife habitat, or as biofuel feedstock. Therefore, their responses to soil contamination by oil and gas drilling and production operation and their potential use in phytoremediation should be evaluated. The current study is only a small step toward this general effort. In this study, we focused on grass species because most of them have extensive fibrous root system, which is desired for hosting soil microbes which contribute to hydrocarbons degradation and reduction in the soil. Another reason is that grass species are relatively easy to establish and require low maintenance (less fertilization and irrigation, infrequent mowing, and less management practices). Lastly, the majority of species used for soil reclamation in the oil and gas exploration areas and abandoned mines in North Dakota are grasses (Rinella et al., 2012). There is not a clear line between reclamation and phytoremediation because many reclaimed areas either still have significant amount of crude oil contamination or maybe vulnerable to such contamination due to the close vicinity to the operation (Aprill and Sims, 1990).

The results from this study did not show a clear difference between native and introduced grass species in their responses to drill cuttings or crude oil contamination. According to the results, native species with moderate tolerance to drill cuttings and crude oil hydrocarbons, such as little bluestem and big bluestem are recommended for use in phytoremediation and reclamation. Also, introduced species may be used for phytoremediation, such as quackgrass and rough bluegrass for drill cuttings contamination, and annual ryegrass and hard fescue for crude oil contamination. However, when using introduced species, care must be taken to avoid invasive ones. For example, Johnsongrass was tolerant to drill cuttings but is invasive and listed as a noxious weed in many states. Downy brome was tolerant in vegetative stage but is listed as noxious weed in North Dakota. Quackgrass, barnyardgrass, and Japanese brome are moderately tolerant to drill cuttings and crude oil, but they are important weeds in field crops of North Dakota. Soil contamination may exert high selection pressure on certain species and create mono stand of invasive species and weeds, which is not desirable for the stability of ecosystem, which include resilience and consistency to persistence (Grant et al., 2009).

There was no clear general trend between annual and perennial grasses with respect to their tolerance to drill cuttings or crude oil. All grass materials used in this study were established from seeds. However, some grass species are primarily propagated via vegetative structures (rhizomes and stolons), and the responses during the establishment using vegetative material may be different from that during seed germination. More research is needed to evaluate the feasibility of using vegetative means to establish grasses in soils contaminated by drill cuttings or crude oil.

4.2. Drill Cuttings and Crude Oil Hydrocarbons

This study tested both drill cuttings and crude oil effects on grass species. However, none of them has a specific characteristic component and the chemical and physical properties are different depending on the sources, especially for drill cuttings (Anoliefo et al., 2006). Crude oil may vary in the types of hydrocarbons and content (Van Epps, 2006). Drill cuttings may vary in content of total petroleum hydrocarbons (TPH), salinity, pH, metals, and other toxic materials in drill mud. The lubricants used in the process contributes to the different composition of the drill cuttings. Lubricants used and their composition are proprietary trade secrets of oil companies (Al-Ansary and Al-Tabaa, 2007; Breuer et al., 2004). As a result, consideration must be given to specific contaminants when choosing grass species for the purposes of phytoremediation and reclamation.

Results found in this study showed that crude oil hydrocarbons reduced seed germination, initial growth during the germination, and biomass during the vegetative stage. In previous studies, crude oil hydrocarbons did not inhibit the sexual reproduction of cereals and foxtail barley (Kisic et al., 2009). Additionally, research showed that significant yield reduction by crude oil hydrocarbons in wheat and maize, but no crude oil hydrocarbons were detected in seeds indicating seeds do not absorb hydrocarbons (Chaineau et al., 1996). Therefore, cereals may be used for remedying soil contaminated solely by crude oil provided it is economically feasible to raise the crops. Drill cuttings may contain metals or other toxic chemicals that accumulate in plants which will render crops grain unusable for food or feed.. Research needs to be done to determine the content of those components in plant tissues and cultural practice has to be established to properly treat the biomass of the grass species used for the remediation of soils contaminated by drill cuttings.

A bigger knowledge gap exists in understanding how both soil and plant are affected by drill cuttings as compared with crude oil. In addition to the complex components in drill cuttings, disposal methods used in the oil industry may also be important. In the case of dispose of drill cuttings in a retaining pond, the leaching into soils nearby or in ground water is of concern (Prantera et al., 1991; Saint-Fort and Ashtani, 2014). Whereas in the case of landfarming (direct application of drill cuttings in farmland), both chemical and physical properties of drill cuttings are important (Prantera et al., 1991). In this study, we did not test the soil physical and chemical properties other than pH and electrical conductivity (EC) as affected by drill cuttings and crude oil hydrocarbons. Nevertheless, hydrophobicity was observed especially at higher concentration of contaminations as shown by decreased water infiltration rate and water drops staying at the surface of soil. Also, the pH and salinity and the effect of other chemicals are confounded as all increase with increasing amounts of drill cuttings in the treatments. The success of a phytoremediation is determined by soil, plants, and environmental conditions, further research is needed to understand the effects of drill cuttings and crude oil on soil properties.

4.3. Phytoremediation

Soil contaminated by petroleum can be remediated through a series of engineering processes when large amounts of contaminants exist in soil (Chaineau et al., 1996; Norris, et al., 1999). Some of these are conducted on site while others require the excavation and removal of soil which is treated in other location (Chaineau et al., 1996). Both *ex situ* and *in situ* processes use either soil treatment systems or leachate/wastewater treatment systems, for instance, thermal treatment, incineration, soil washing, chemical extraction, land farming, composting, bioreactors, bioremediation, and phytoremediation. However, the selection of the technology has to be based on the regulatory demands by EPA and local government (e.g. total petroleum hydrocarbon amount), the properties of the contaminants, site characteristics, time, and cost (Van Epps, 2006).

The cost of these treatment technologies varies with the phytoremediation as the lowest one. For example, phytoremediation cleanup per cubic meter was $648 less than excavation and incineration (Rock and Sayre, 1998). The maximum TPH content and salinity levels set by EPA (Van Epps, 2006) were in the range of concentrations used in this study. Our results indicated that annual ryegrass can reduce about 12.8 m^3 of hydrocarbons per hectare. The cost of planting and maintaining for some native grass species is approximately $560 ha^{-1} (Doxon et al., 2011).

4.4. Chemical, Biochemical, and Physiological Aspects of Grass Response to Drill Cuttings and Crude Oil

Grass seed germination consists of multiple chemical and biochemical reactions. Seeds of grasses species that have dormancy were pretreated according to the ISTA procedures. Larger variations were observed in germination at two weeks after the treatment by drill cuttings or crude oil. Since the germination status did not change one month beyond the two-week time period recommended in ISTA procedures, it was not likely that the contaminants induced secondary dormancy in seeds. Direct toxicity or permeability of water, air, and hydrocarbons into seeds may be more responsible for the inhibition of germination. Maize and cereal crops showed less germination reduction compared with other grass species, indicating seed size may also be a factor (Mouissie et al., 2005). However, the mechanism of germination inhibition requires further study.

Hydrocarbons content in soil from drill cuttings or crude oil decreased after growing grass species on it. Annual ryegrass and barley were among the top of nine species tested for their ability of facilitating the reduction of hydrocarbons in soil. According to previous reports, direct uptake by plants was not responsible for the reduction in hydrocarbon content (Miller and Pesaran, 1980; Saint-Fort and Ashtani, 2014). Microbial activity was reported as the major mechanism of hydrocarbon reduction (Fan et al., 2014), and most likely it was responsible for hydrocarbon reduction in this study. Since we used controlled irrigation and no significant amounts of leaching were observed, other mechanisms such as volatilization of hydrocarbons (Fine et al., 1997) from the soil surface may also have influenced the final content of hydrocarbons. The initial amount of volatile components was probably not high because the contaminated soil was used after exposed to air until the odor of hydrocarbons was no longer detected; however, odorless volatile compounds may exist (Campanella et al., 2003). Direct absorption of hydrocarbon by the activated charcoal layer used was not likely because the particle sizes of the activated charcoal were larger than 2 mm and at this size there is no significant capillary rise from the root zone into charcoal layer (Hanks, 1992).

The biological reduction of hydrocarbons in the root zone is a complicated process. Different chemical active groups exist in the drill cutting shown in the FTIR spectra of the contaminated soil after phytoremediation. Each chemical group has a different fate in the contaminated soil. However, more research is needed to understand the fate and dynamics of hydrocarbons in soil and identify the actual products from the degradation.

In general, the results found in this study have confirmed that grass species have different levels of tolerance to drill cuttings and crude oil contamination in soil as reported previously. Little bluestem and big bluestem showed moderate tolerance to drill cuttings and crude oil in this study and are native to North Dakota. Therefore, they are recommended for reestablishing vegetation in soil contaminated by oil and gas drilling operations. Annual ryegrass and rough bluegrass could be used to accelerate the degradation of hydrocarbons in soil. Cereal crops showed tolerance to crude oil and drill cuttings, but are only recommended when no toxic materials are accumulated in plants or seeds before their normal use as feed.

4.5. References

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